Molecular epidemiology of Dengue in Thailand and Laos

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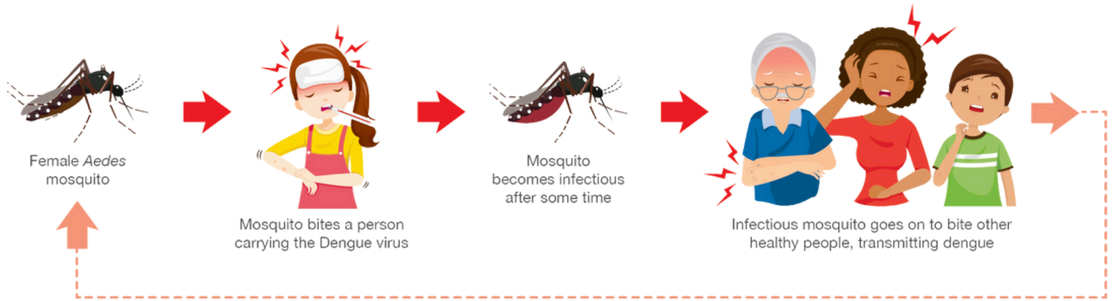
Background on Dengue

DENGUE FEVER



- Causes **a hundred million** dengue cases annually, with significant morbidity and mortality.

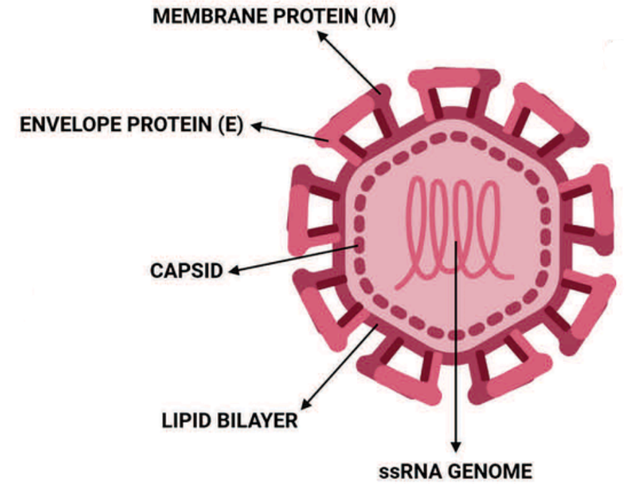
- **No specific antiviral drugs** and **vaccine provide limited** or incomplete.

Factors

- Climate change
- Large urban center
- Globalization
- Limited in surveillance and reporting

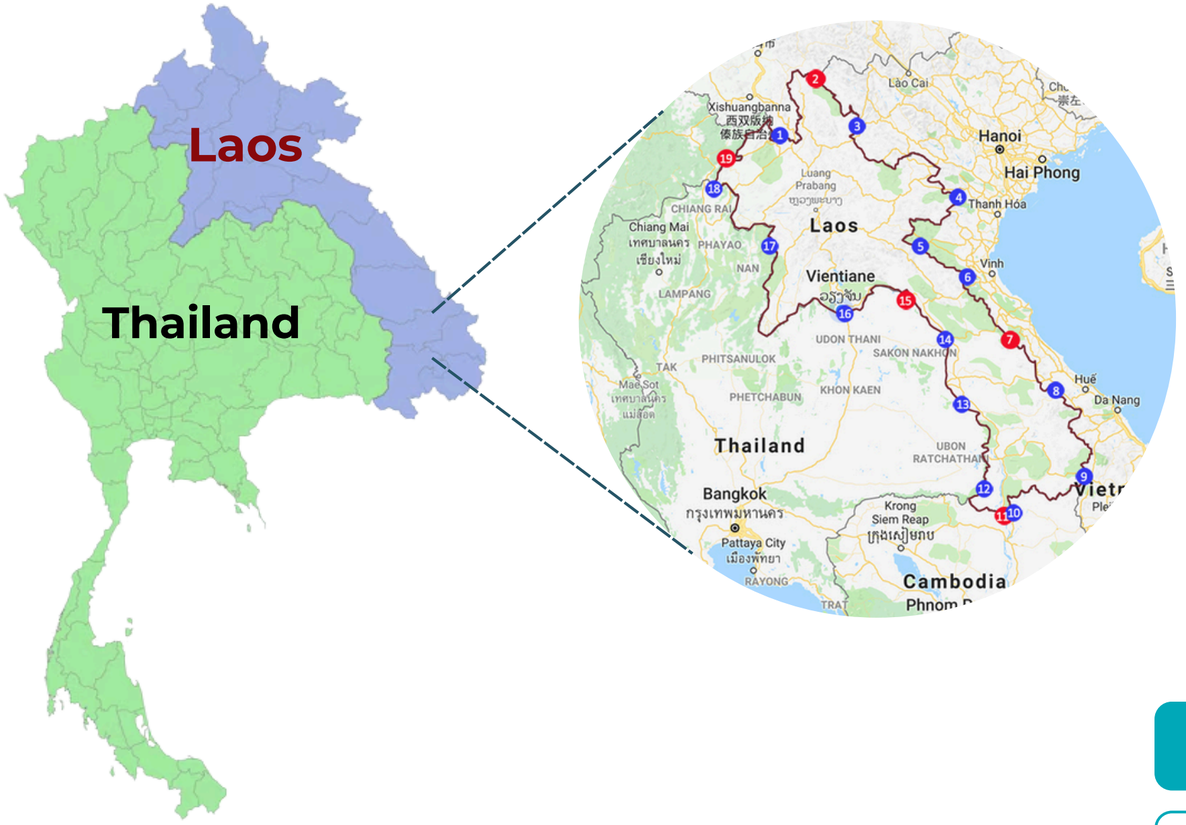
DENGUE VIRUS

- Genus : *Flavivirus*
- Family : *Flaviviridae*
- Genome : +ssRNA enveloped virus
- 4 serotype : DENV-1 to DENV-4



Ruvalcaba-Hernández et al., 2025

Dengue in Thailand and Laos



Extensive land border

Severe dengue outbreaks

Hyperendemic with 4 serotype

THAILAND	LAOS
1st DHF outbreak in 1958	1st DHF outbreak in 1985

Dengue in Thailand and Laos

PLOS ONE

RESEARCH ARTICLE

Phylogenetic analysis revealed the co-circulation of four dengue virus serotypes in Southern Thailand

Rodolphe Hamel[1,2], Pornapat Surasombatpattana[3], Sineewanlaya Wichit[4], Alexandra Dauvé[5], Celeste Donato[6], Julien Pompon[1,7], Dhanasekaran Vijaykrishna[6,7], Florian Liegeois[1], Ronald Morales Vargas[5], Natthanej Luplertlop[2], Dorothée Missé[1]*

DENV-3 Genotype III were closely grouped in a monophyletic lineage with strains isolated from Laos.

Some circulating DENV-1 in Laos may have originated from viruses circulating in Thailand.

OPEN ACCESS Freely available online

PLOS NEGLECTED TROPICAL DISEASES

An Epidemic of Dengue-1 in a Remote Village in Rural Laos

Audrey Dubot-Pérès[1,2,3]*, Phengta Vongphrachanh[4], Justin Denny[5], Rattanaphone Phetsouvanh[2,3], Singharath Linthavong[6], Bounthanom Sengkeopraseuth[4], Amphai Khasing[4], Vimattha Xaythideth[4], Catrin E. Moore[2,3], Manivanh Vongsouvath[2], Josée Castonguay-Vanier[2,3], Bountoy Sibounheuang[2], Thaksinaporn Taojaikong[2†], Anisone Chanthongthip[2], Xavier de Lamballerie[1], Paul N. Newton[2,3]

1 UMR_D 190 Emergence des Pathologies Virales, Aix-Marseille Université, IRD French Institute of Research for Development, EHESP French School of Public Health, Marseille, France, 2 Lao-Oxford-Mahosot Hospital - Wellcome Trust Research Unit (LOMWRU), Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic, 3 Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Churchill Hospital, Oxford, United Kingdom, 4 National Centre for Laboratory and Epidemiology, Ministry of Health, Vientiane, Lao People's Democratic Republic, 5 World Health Organization – Lao People's Democratic Republic, Vientiane, Lao People's Democratic Republic, 6 Xayabury Provincial Health Department, Xayabury, Lao People's Democratic Republic

Dengue in Thailand and Laos

DENV circulating in Lao PDR are part of a broader regional transmission network involving Thailand.

microorganisms

MDPI

Article

Molecular Epidemiology of Dengue Viruses in Lao People's Democratic Republic, 2020–2023

Cécile Troupin [1], Kedkeo Intavong [1], Somphavanh Somlor [1], Souksakhone Viengphouthong [1], Sitsana Keosenhom [1], Thep Aksone Chindavong [1], Phaithong Bounmany [1], Longthor Vachouaxiong [1], Thonglakhone Xaybounsou [1], Chittaphone Vanhnollat [1], Phonepadith Khattignavong [2], Darouny Phonekeo [3], Bouaphanh Khamphaphongphane [4], Phonepadith Xangsayarath [5], Vincent Lacoste [1,†], Philippe Buchy [3,*] and Gary Wong [1,*]

International Journal of *Environmental Research and Public Health*

MDPI

Article

Development and Comparison of Dengue Vulnerability Indices Using GIS-Based Multi-Criteria Decision Analysis in Lao PDR and Thailand

Sumaira Zafar [1,*], Oleg Shipin [1], Richard E. Paul [2], Joacim Rocklöv [3], Ubydul Haque [4], Md. Siddikur Rahman [5,6], Mayfong Mayxay [7,8,9], Chamsai Pientong [5], Sirinart Aromseree [5], Petchaboon Poolphol [10], Tiengkham Pongvongsa [11], Nanthasane Vannavong [12] and Hans J. Overgaard [5,13]

Dengue vulnerability patterns in Thailand and Lao PDR are shaped by similar environmental and socioeconomic drivers.

Evidence of transmission linkage or cross-border viral spread.

Dengue in Thailand and Laos

Dengue viruses circulating in Lao PDR are part of a broader regional transmission network involving Thailand

microorganisms

MDPI

Article

Molecular Epidemiology of Dengue Viruses in Lao People's Democratic Republic, 2020–2023

Previous studies were largely limited to analyses of the envelope (E) gene and lacked comprehensive molecular epidemiological characterization.

Article

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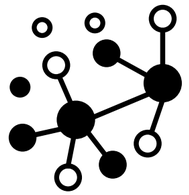
Evidence of transmission linkage or cross-border viral spread

Molecular epidermiology

Genetic data + **Epidemiological information**

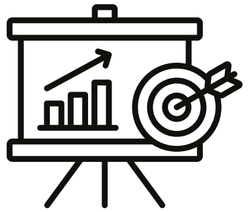
Relationships



Identify genetic relatedness among viral strains, define clusters and transmission networks.

01

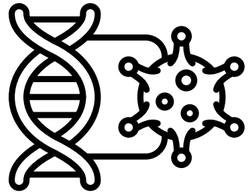
Strains shifts



Detect genotype replacement over time, reflect viral fitness and population immunity

02

Evolutionary



Estimate divergence times and MRCA, reconstruct viral evolutionary history.

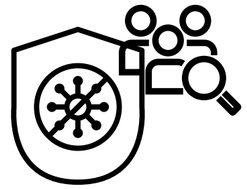
03

Transmission

Infer viral movement, identify transmission routes, hubs and directionality

04

Outbreak



Distinguish repeated introductions vs local spread and link viral genetics to epidemic events

05

Objective

To investigate the molecular epidemiology and phylogeographic relationships of dengue virus in Thailand & Laos

Specific objectives

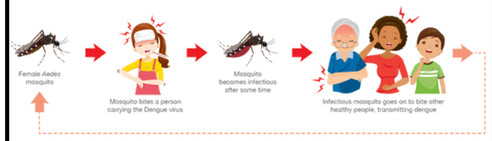
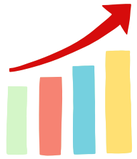
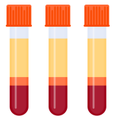
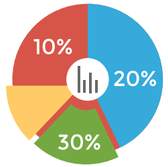
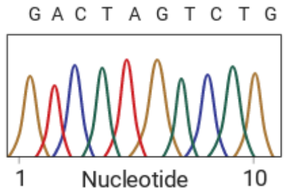
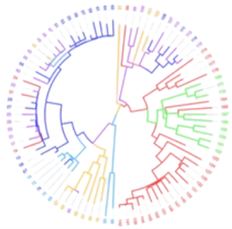
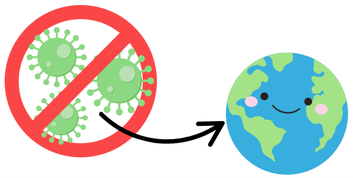
- To identify and characterize DENV serotypes and genotypes circulating in Thailand and Laos
- To assess the genetic diversity and phylogenetic relationships of DENV isolates from both countries.
- To reconstruct phylogeographic patterns and infer possible routes of virus movement between Thailand and Laos.
- To evaluate the extent of gene flow and viral exchange across the Thailand–Laos border regions.

Hypothesis

1. DENV circulating at the Thailand–Laos border exhibits high genetic similarity due to frequent cross-border introductions and the exchange of viral lineages.

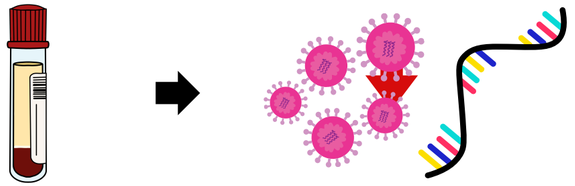
2. Recent DENV genomes differ from previous, reflecting measurable evolutionary changes over time.

Conceptual framework

Problem	Process	Output	Outcome	Impact
Dengue  Emerging and re-emerging disease  Climate change, urban center, globalization ⬇ **Need to understand the dynamics of dengue virus** ⬇ **Molecular epidemiology**	1. Sample collection  2. Dengue detection and enrichment  3. Dengue sequencing  4. Bioinformatic analysis 	Proportion of circulating dengue virus in Thailand and Laos  Dengue sequences from Thailand and Laos  Phylogenetic and phylogeography map 	Understand the relationship of Dengue Thailand and Laos  Understand dengue dynamics in Thailand and Laos  Identify hotspot of transmission 	**Strong scientific, gene flow and evolutionary of DENV** **Reduction of cross-border dengue transmission** **Support for vaccine and prevention strategies** **Predicting outbreaks and reduced disease burden** 

Study design

1. Molecular Testing



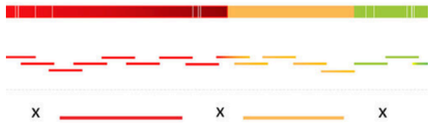
Viral RNA extraction

DENV detection and serotyping

Proportion circulating dengue in Thai/Laos

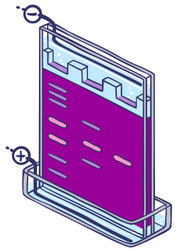
2. DENV-WGS

Select positive sample



Whole-genome enrichment
(overlapping amplicons approach)

Gel purification



Sanger sequencing

3. Bioinformatics

Create data set
(Study sequences + published sequences)

Identify DENV lineages using Maximum likelihood

Validate temporal signal

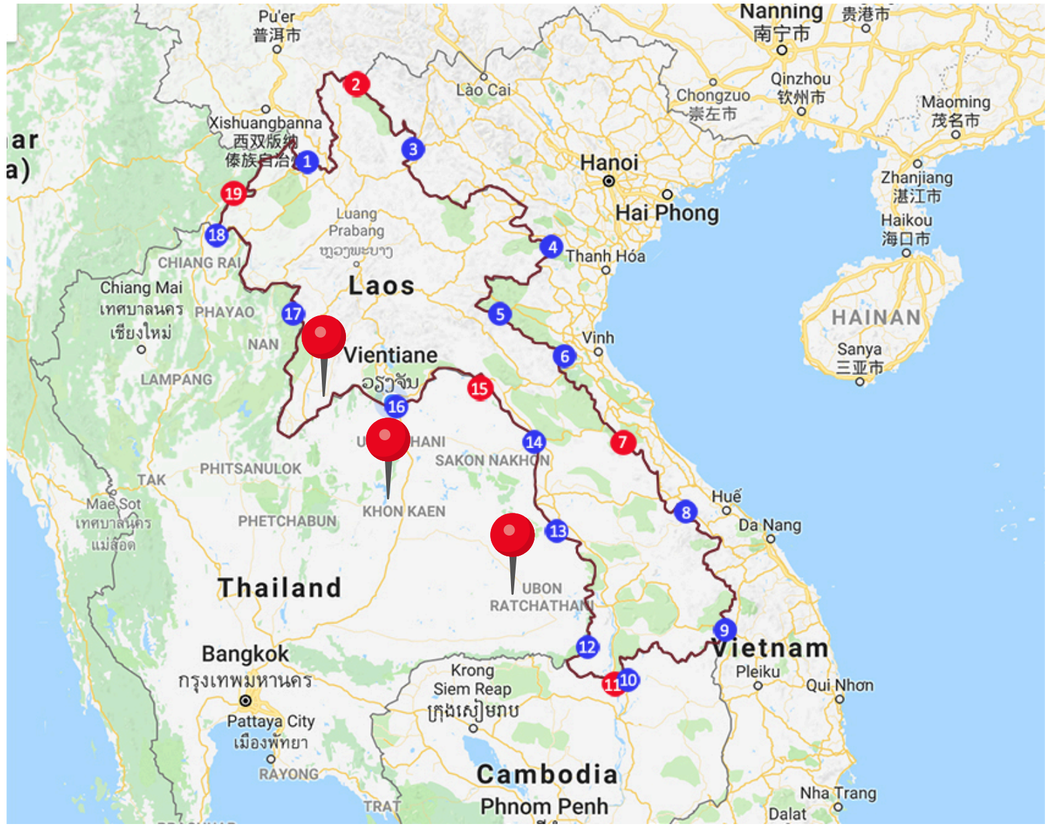
Bayesian analyses

- Evolutionary rate/tMRCA
- Transmission rate/map

Study site

Objective

To ensure representative coverage of dengue transmission across area and cross-border settings.



UBON RATCHATHANI

KHON KAEN

VIENTIANE

High incident

All three sites are major urban centers with high human mobility.
Ubon Ratchathani and Vientiane serving as cross-border hubs.

Sample size

Objective

To provide sufficient statistical power for downstream analyses.

Clinical sample

EPITOOLS → Assuming 95% confidence level → **140 samples**

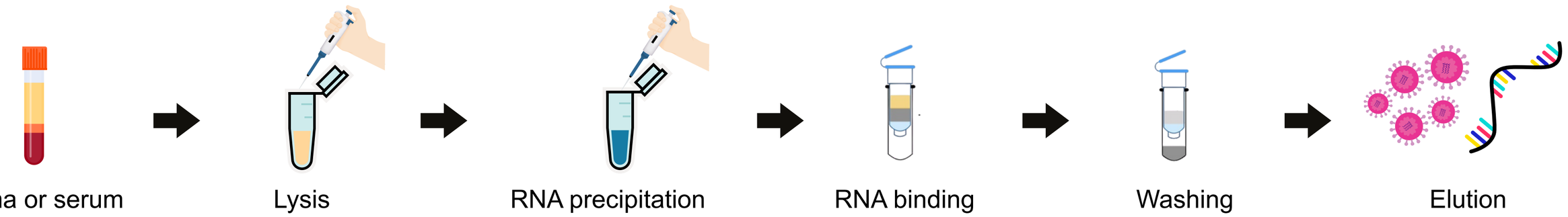
Phylogenetic analysis

Phylosamp package → Assuming 95% confidence level → **10 sequences**

Viral RNA extraction

Objective To isolate high-quality and intact viral RNA suitable for downstream molecular analyses.

METHOD



Ubon2019

Obtained 300 samples

Ubon2019

Extracted 100 samples

Laos2019

Obtained 200 samples

Future work

- **RNA extraction**

  Continue sample extraction from Ubon Ratchathani and Lao PDR (2019).

- **DENV detection and serotyping**

  Initiate DENV detection and serotyping.

- **EC application**

  Apply for ethical clearance (EC) to collect samples from Ubon Ratchathani (2025).

- **Proposal examination**

Thesis plan

Activities	2025		2026				2027	
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
1. Literature Review and Planning								
2. Proposal Writing								
3. Proposal Examination								
4. EC Application (Thailand 2025)								
Part I Molecular testing								
5. Viral RNA extraction								
6. DENV detection and serotyping								
Part II Whole-genome sequencing								
7. Select positive DENV sample from Part I								
8. Design the primer for whole-genome of each serotype								
9. Whole-genome DENV enrichment								
10. Whole-genome sequencing								
Part III Bioinformatics analyses								
11. Create dataset of DENV sequences								
12. ML and Bayesian anlyses								
13. Proceeding								
14. Thesis defense examination								

Done

On process

Future work

Acknowledgement

Advisor

Assoc.Prof.Dr.Supranee Phanthanawiboon

SP lab







THANK YOU FOR YOUR ATTENTION