# Thesis progression

Thesis title: Molecular epidemiology of Dengue in Thailand and Laos

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## 1. Introduction

Dengue fever is an increasingly important mosquito-borne viral infection that poses a significant public health burden especially tropical and subtropical regions, particularly Southeast Asian. The disease is now endemic and affecting over 100 countries (1). Symptomatic cases of dengue infection present with an acute illness characterized by flu-like symptoms, including fever and headache, called dengue fever and in some cases progresses to severe dengue characterized by abdominal pain, hemorrhage, and circulatory collapse, which can lead to death. Dengue fever is caused by a dengue virus as a member of the genus *Flavivirus* consisting of 4 serotypes (DENV1-4). The virus is primarily transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes (1). Over the past few years, dengue has been an upsurge of dengue cases and deaths reported globally, which is characterized by a significant increase in the number, scale and simultaneous occurrence of multiple outbreaks, spreading into regions previously unaffected by dengue (1). Dengue epidemics have been widely reported across Asia, South-East Asian countries are particularly vulnerable regions to climate risks (2) including Thailand and Lao People’s Democratic Republic (Laos). Increased dengue risk is generally associated with climate change, rapid urban growth, and increasing global connectivity, which have contributed to the wider spread and greater diversity of the dengue virus. (3–5).

Thailand and Laos have experienced severe dengue outbreaks (6–8). In Thailand, where dengue is hyperendemic with all four serotypes co-circulating, approximately 20,000–140,000 cases are reported annually, and the entire population remains at risk of infection

(9). In Laos, dengue outbreaks recur periodically, affecting multiple provinces(10). Thailand shares extensive land borders with Laos, where numerous border-crossing points facilitate frequent cross-border population movement. Although dengue is a major public health threat in Thailand and Laos, the evolutionary characteristics of the virus and the direct relationships between dengue viruses circulating in the two countries remain poorly understood. The lack of comprehensive phylogeographic data limits understanding of how viral genotypes are introduced into and persist within countries, as well as how gene flow occurs across national borders, thereby constraining evolutionary analyses. These gaps hinder the ability to predict viral evolution, inform vaccine development, and implement region-specific control strategies.

Previous studies have shown that dengue virus strains from Thailand clustered closely within monophyletic lineages together with strains isolated from Laos, which may indicate cross-border viral introductions and possible circulation of shared viral lineages between neighboring countries (5,11–13). However, these studies were largely limited to analyses of the envelope (E) gene and lacked comprehensive molecular epidemiological characterization. Whole-genome analyses and direct comparisons of dengue viruses circulating in both countries would provide deeper insights into viral evolutionary dynamics. Improving understanding of these evolutionary dynamics is essential for overcoming the limitations of current dengue risk assessments and enabling more strategic long-term disease management. Therefore, the overall purpose of this study was to investigate the molecular epidemiology and phylogeographic patterns of dengue virus between Thailand and Laos to support the development of effective surveillance and control strategies.

**2. Hypothesis**

Dengue virus populations circulating along the Thailand–Laos border are hypothesized to be genetically interconnected, reflecting frequent cross-border introductions and the exchange of viral lineages. In addition, contemporary DENV sequences are expected to differ from those collected in earlier periods indicating ongoing evolutionary changes over time. Comparative analyses with historical sequences obtained from public databases will enable assessment of viral evolutionary dynamics, including potential alterations related to virulence and immune evasion.

## 3. Objective

### 3.1 General objective

To investigate the molecular epidemiology and phylogeographic patterns of dengue virus in Thailand and Laos.

### 3.2 Specific objectives

3.2.1 To assess the genetic diversity and phylogenetic relationships of DENV isolates from both countries.

3.2.2 To evaluate the extent of gene flow and viral exchange across the Thailand–Laos border regions.

3.2.3 To reconstruct phylogeographic patterns and infer possible routes of virus movement between Thailand and Laos.

## 4. Anticipated outputs

4.1 Proportion of circulating dengue in Thailand and Laos.

4.2 Dengue sequences from Thailand and Laos.

4.3 Phylogenetic trees and phylogeographic maps of dengue.

## 5. Anticipated outcomes

5.1 Improved understanding of dengue virus circulation and evolutionary dynamics in Thailand and Laos.

5.2 Improved understanding of the epidemiological and evolutionary relationships of dengue virus between Thailand and Laos.

5.3 Identification of dengue transmission hotspots.

5.4 Generation of evidence to inform vaccine development and targeted control strategies.

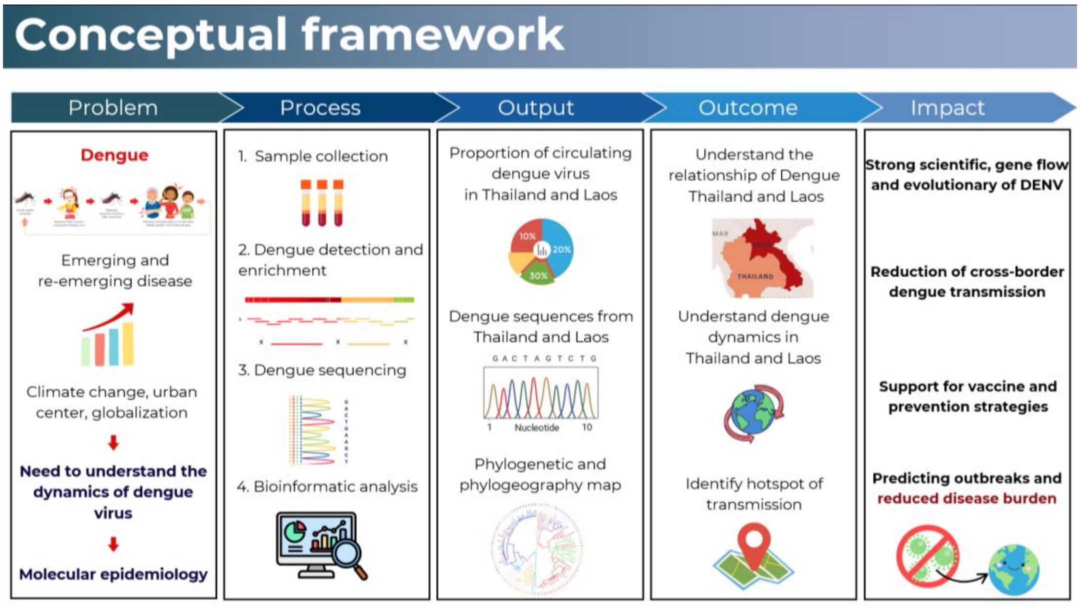
5.5 Enhanced disease surveillance and public health planning.

## 6. Impact

The molecular epidemiology of dengue in Thailand and Laos will support a deeper scientific understanding of dengue virus gene flow, evolutionary dynamics, and transmission patterns between Thailand and Laos through comprehensive genomic and phylogeographic analyses. By elucidating cross-border viral movement and lineage exchange, the findings will provide evidence-based insights that can inform targeted public health interventions aimed at reducing cross-border dengue transmission. In addition, the generated genomic data and evolutionary insights will strengthen the scientific foundation for dengue vaccine development and prevention strategies. Ultimately, improved understanding of dengue virus evolution and spread will enhance outbreak prediction capacity and contribute to reducing the overall dengue disease burden in the region.

## 7. Conceptual framework

This study will employ a combination of molecular and computational techniques. Serum or plasma samples will be collected from suspected dengue cases and confirmed dengue patients across Thailand and Laos. Viral RNA will be extracted using commercial kits according to the manufacturers' instructions. Dengue virus infection and serotypes will be confirmed by RT-qPCR. Viral genes will then be amplified and sequenced to characterize viral genotypes and lineages. Phylogenetic and phylogeographic analyses will be performed using bioinformatic and Bayesian approaches to infer evolutionary dynamics, geographic origins, and circulation patterns. Molecular findings will be integrated with epidemiological data to provide insights into the introduction, spread, and persistence of dengue virus genotypes, thereby supporting the development of targeted surveillance and control strategies.




## 8. Study design

This study will be conducted in three major parts including: (I) Sample preparation and molecular testing, (II) Dengue whole-genome sequencing and (III) Bioinformatics analysis.

(I) Sample preparation and Molecular testing

This part will be performed to identify dengue virus infection and determine circulating dengue virus serotypes in samples collected from Thailand and Lao PDR. Serum or plasma samples will be collected from dengue-infected or dengue-suspected patients in both countries. Viral RNA will be extracted from clinical samples using commercial extraction kits. Dengue virus infection will be confirmed and serotyping.

(II) Dengue whole-genome sequencing

Dengue-positive samples from each serotype with RNA quality and viral load will be selected for whole-genome sequencing. Viral RNA will be reverse-transcribed into complementary DNA (cDNA) and amplified using dengue-specific primers to generate overlapping amplicons spanning the entire genome. Purified amplicons will be sequenced using the Sanger method, and sequencing quality and genome completeness will be evaluated to ensure suitability for downstream analyses.

(III) Bioinformatics analysis.

Multiple sequence alignment will be performed using newly generated sequences together with reference and publicly available dengue virus genomes. Phylogenetic analyses will be carried out using maximum likelihood and Bayesian inference methods. Time-resolved phylogenetic and phylogeographic analyses will be applied to investigate viral evolution, lineage movement, gene flow and cross-border transmission dynamics between the two countries.

**9. Materials and methods**

**9.1 Sample**

Plasma or serum samples will be collected from dengue-infected or dengue-suspected patients in Thailand and Laos during 2019 and 2025. All clinical specimens were stored at −80 °C until further processing.

Sample sizes were estimated using established statistical tools, including EPITOOLS and Phylosamp implemented in R. EPITOOLS was used to estimate the number of clinical samples required based on a single-proportion (apparent prevalence) model for dengue virus detection, resulting in a total of 139 clinical samples. In parallel, Phylosamp was used to estimate the minimum number of viral genome sequences required for phylogenetic analyses. Using the translink_samplesize() function, a minimum of 10 high-quality genome sequences were estimated to be sufficient for detecting phylogenetic transmission linkages.

**9.2 RNA extraction**

Viral RNA will be extracted from plasma or serum samples using commercially available extraction kits following the manufacturers' protocols. The eluted RNA will be aliquoted to minimize freeze–thaw cycles and stored at −80 °C until further analysis.

**9.3 Dengue detection and serotyping**

Viral RNA will first be reverse-transcribed into complementary DNA (cDNA) using dengue virus–specific primers. Dengue virus detection will then be performed using conventional polymerase chain reaction (PCR) according to published primers and protocols (14). Samples positive for dengue virus will subsequently be subjected to serotyping by quantitative real-time PCR (qPCR) using published primers (15).

**9.4 Whole-genome sequencing**

Positive samples from each dengue serotype will undergo whole-genome sequencing (WGS) using an overlapping amplicon-based enrichment approach, with primer sets designed to cover the entire viral genome. Successfully amplified amplicons will be gel-purified and sequenced to generate near-complete dengue virus genomes, which will be used to assess the genetic diversity of circulating dengue viruses in Thailand and Laos.

**9.5 Bioinformatics analyses**

Newly generated dengue virus genome sequences will be analyzed together with publicly available reference sequences retrieved from international databases, including GenBank. Multiple sequence alignment will be performed using MAFFT, and alignments will be manually inspected and curated to ensure sequence quality. Phylogenetic analyses will be conducted using both maximum likelihood (ML) and Bayesian inference approaches. ML phylogenetic trees will be reconstructed using appropriate nucleotide substitution models with statistical support assessed by bootstrap resampling. Bayesian phylogenetic analyses will be performed to infer evolutionary relationships and temporal dynamics, including estimation of divergence times where applicable. These analyses will be used to investigate the genetic diversity, evolutionary relationships, and transmission patterns of dengue viruses circulating in Thailand and Laos.

## 10. References


1. WHO, 2024. Dengue - Global situation [Internet]. [cited 2026 Jan 28]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON518

2. Bowen KJ, Ebi KL. Health risks of climate change in the World Health Organization South-East Asia Region. WHO South-East Asia J Public Health. 2017 Sep;6(2):3.

3. Lee SA, Economou T, Barcellos C, Catão R, Carvalho MS, Lowe R. Effect of climate change, connectivity, and socioeconomic factors on the expansion of the dengue virus transmission zone in 21st century Brazil: an ecological modelling study. Lancet Planet Health. 2021 Apr 1;5:S14.

4. Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol. 2014 Mar;22(3):138–46.

5. Phadungsombat J, Nakayama EE, Shioda T. Unraveling Dengue Virus Diversity in Asia: An Epidemiological Study through Genetic Sequences and Phylogenetic Analysis. Viruses [Internet]. 2024 Jun 28 [cited 2026 Feb 2];16(7). Available from: https://www.mdpi.com/1999-4915/16/7/1046

6. MOPH Thailand, 2013, MOPH Thailand. Epidemiology. Annual Epidemiological Surveillance Report [Internet]. [cited 2026 Feb 2]. Available from: https://apps-doe.moph.go.th/boeeng/annual/Annual/AESR2013/table.php

7. MOPH Thailand, 2015. MOPH Thailand. [cited 2026 Feb 2]. Annual Epidemiological Surveillance Report 2015. Available from: https://apps-doe.moph.go.th/boeeng/annual/Annual/AESR2015/sum_aesr.php

8. Vannavong N, Seidu R, Stenström TA, Dada N, Overgaard HJ. Dengue-like illness surveillance: a two-year longitudinal survey in suburban and rural communities in the Lao People's Democratic Republic and in Thailand. West Pac Surveill Response J WPSAR. 2019;10(1):15–24.

9. Nonyong P, Ekalaksananan T, Phanthanawiboon S, Aromseree S, Phadungsombat J, Nakayama EE, et al. Dengue virus in humans and mosquitoes and their molecular characteristics in northeastern Thailand 2016-2018. PloS One. 2021;16(9):e0257460.

10. Mayxay M, Cui W, Thammavong S, Khensakhou K, Vongxay V, Inthasoum L, et al. Dengue in peri-urban Pak-Ngum district, Vientiane capital of Laos: a community survey on knowledge, attitudes and practices. BMC Public Health. 2013 May 3;13:434.

11. Phylogenetic analysis revealed the co-circulation of four dengue virus serotypes in Southern Thailand | PLOS One [Internet]. [cited 2026 Feb 2]. Available from: https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0221179&utm_source=chatgpt.com

12. Dubot-Pérès A, Vongphrachanh P, Denny J, Phetsouvanh R, Linthavong S, Sengkeopraseuth B, et al. An Epidemic of Dengue-1 in a Remote Village in Rural Laos. PLoS Negl Trop Dis. 2013 Aug 8;7(8):e2360.

13. Troupin C, Intavong K, Somlor S, Viengphouthong S, Keosenhom S, Chindavong TA, et al. Molecular Epidemiology of Dengue Viruses in Lao People's Democratic Republic, 2020–2023. Microorganisms [Internet]. 2025 Feb 1 [cited 2026 Feb 3];13(2). Available from: https://www.mdpi.com/2076-2607/13/2/318

14. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. 1992 Mar;30(3):545–51.

15. 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 Jun;41(6):2408–16.