

Phylogenetic and Spatial Dynamics of Rabies Virus in Thailand, 1998–2014: A Retrospective Analysis Using MicrobeTrace

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Abstract

Objectives: Rabies remains an important zoonotic disease in Thailand despite long-standing vaccination programs. Understanding long-term viral circulation patterns is essential to support surveillance and control.

Methods: We conducted a retrospective molecular epidemiological analysis of rabies virus (RABV) in Thailand using 307 nucleotide sequences collected between 1998–2002 and 2011–2014. Sequences were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT). Phylogenetic analysis was performed using the neighbor-joining method in Unipro UGENE software, and the resulting trees were visualized using iTOL. Molecular networks were constructed in MicrobeTrace using a genetic distance threshold of 0.015. Spatial and temporal patterns were mapped using QGIS.

Results: Phylogenetic and molecular network analyses consistently classified RABV into four genetic clusters. Clusters 1–3 belonged to the Southeast Asia clade, while Cluster 4 was related to the China clade. Cluster 1 showed widespread geographic distribution, persistent circulation, and high network connectivity, indicating endemic maintenance. Cluster 2 was detected throughout the study period across multiple regions, but exhibited network degree values below the median, suggesting more fragmented transmission. Cluster 3 was detected only during the early study period (1998–2002), indicating limited circulation. Cluster 4 emerged during the later period (2011–2014) and was geographically confined to northern and upper northeastern border provinces.

Public Health Recommendations: This study highlights the value of integrating phylogenetic, molecular network, and spatial analyses to better characterize rabies transmission dynamics. The findings support strengthening targeted surveillance in high connectivity and border areas, sustaining mass dog vaccination, and enhancing cross-border collaboration within a One Health framework.

Keywords: MicrobeTrace, molecular epidemiology, rabies virus, Thailand

Introduction

Rabies is a fatal zoonotic disease caused by rabies virus (RABV), a negative-sense, single-stranded RNA virus in the genus *Lyssavirus*, family *Rhabdoviridae*.¹ Once clinical symptoms develop, the disease is almost invariably fatal. Although effective vaccines are available, rabies remains endemic in many countries, including Thailand, where

domestic dogs are the primary reservoir and source of human infection.^{2,3}

In Thailand, rabies control programs include annual mass vaccination of dogs and cats, conducted by local administrative organizations between January and April, alongside stray animal population management by provincial livestock officers and public health interventions



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led by the health sector. However, heterogeneity in vaccination coverage and dog population structure may influence transmission patterns. Therefore, understanding long-term viral circulation is essential to support rabies surveillance and control under a One Health framework.⁴

Molecular epidemiology has become an essential tool for understanding rabies transmission dynamics, identifying endemic viral lineages, and detecting introductions from outside affected areas.^{5,6} Previous studies in Thailand have demonstrated that rabies virus circulation is characterized by multiple co-circulating lineages and long-term endemic maintenance within dog populations. A nationwide study identified two major phylogroups (TH1 and TH2) within the Southeast Asia clade, showing distinct geographic distributions and suggesting virus spread linked to human-mediated dog movement.⁷ At a finer spatial scale, complex genetic structures with multiple co-circulating clades have been observed in urban settings such as Bangkok, suggesting frequent viral movement and localized transmission dynamics.⁸ In addition, studies using complete nucleoprotein gene analysis have identified the coexistence of Southeast Asia and China-associated lineages in Thailand, particularly in border areas, indicating potential cross-border transmission and regional connectivity.⁹

At the regional level, rabies virus dynamics in Southeast Asia are influenced by cross-border connectivity. Studies in neighboring countries have shown that circulating rabies viruses are genetically related across national boundaries, reflecting historical viral exchange within the region.¹⁰ These findings highlight the importance of considering transboundary transmission when interpreting rabies epidemiology in Thailand. Despite these advances, most studies have focused on phylogenetic analysis alone or limited geographic scales. National-level analyses integrating phylogenetic, molecular network, and spatial approaches over extended time periods remain limited.

MicrobeTrace is a web-based platform designed to visualize genetic relatedness and transmission networks, providing additional epidemiological insight beyond traditional phylogenetic analysis.¹¹ Therefore, this study aimed to investigate the phylogenetic and spatial dynamics of rabies virus in Thailand during 1998–2014 by integrating phylogenetic, molecular network, and spatial analyses to better understand long-term transmission patterns and support rabies control under a One Health framework.

Methods

Data Sources

Rabies virus nucleotide sequences were obtained from GenBank and previously published surveillance studies conducted in Thailand between 1998–2002 (7 human, 177 dog, 6 cat and 2 cattle sequences) and 2011–2014 (115 dog sequences), with accompanying metadata on host species, collection date, and geographic location.^{7,9,12} In total, 307 sequences from both human and animal cases were included in the analysis.

Molecular Analysis

Sequences were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT).¹³ Phylogenetic trees were constructed using the neighbor-joining method under the Kimura 2-parameter nucleotide substitution model in Unipro UGENE software, with bootstrap analysis (1,000 replicates) to assess node support. The trees were subsequently visualized using iTOL.^{7,14–16}

Network Analysis

A genetic distance threshold of 0.015 was applied to define network links.¹⁷ Network degree values were calculated, and the median and 75th percentile were used as relative thresholds to interpret viral connectivity.

Spatial Analysis

Geographic information, including province and year of detection, was integrated with molecular data, and mapped using QGIS. Spatial patterns were examined across two periods, 1998–2002 and 2011–2014, based on the available data sources.

Results

Phylogenetic analysis classified RABV sequences into four genetic clusters. Clusters 1–3 were associated with the Southeast Asia clade, whereas Cluster 4 was genetically related to the China clade, with strong bootstrap support (100%), indicating a distinct evolutionary origin (Figure 1).

Molecular network analysis using MicrobeTrace identified four clusters with distinct connectivity patterns. Cluster 1 comprised 206 sequences, Cluster 2 included 56 sequences, Cluster 3 contained 20 sequences, and Cluster 4 consisted of 25 sequences. These clusters were consistent with those identified in the phylogenetic tree analysis (Figure 2).

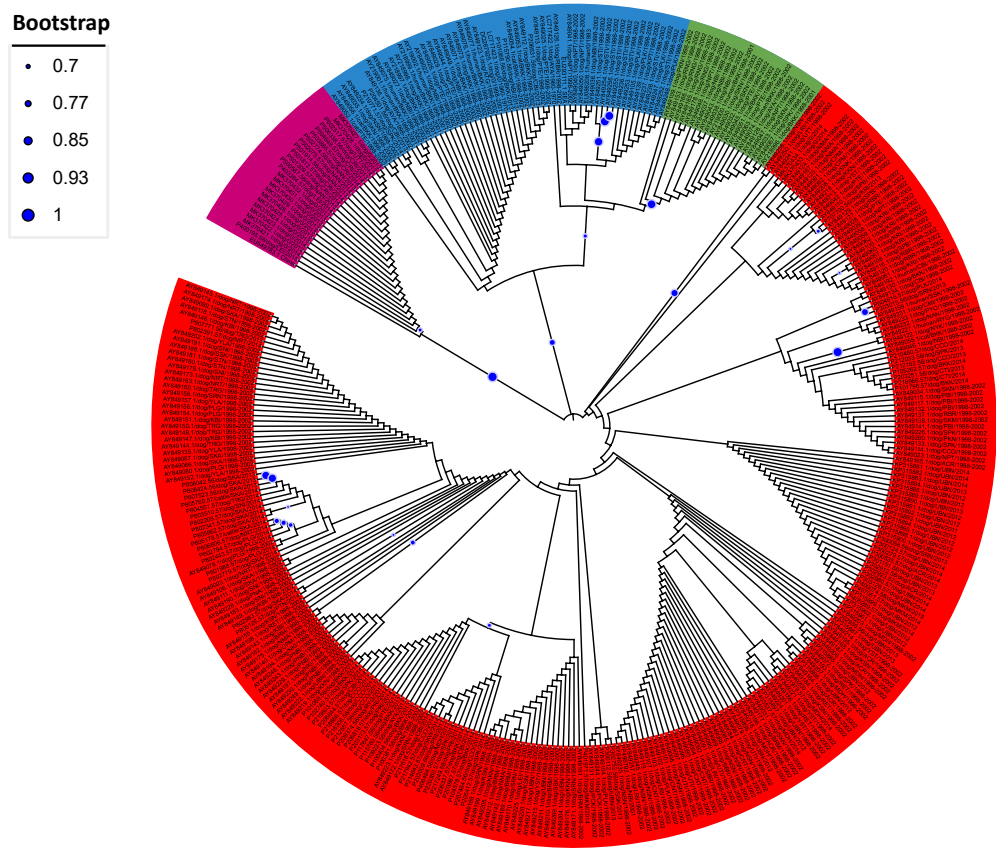


Figure 1. Phylogenetic tree of rabies virus isolates detected in Thailand during 1998–2014, constructed using the neighbor-joining method, with bootstrap support values $\geq 70\%$ (0.7) shown at the nodes. The sequences were grouped into four genetic clusters: Cluster 1 (red), Cluster 2 (blue), and Cluster 3 (green), which belong to the Southeast Asia lineage, and Cluster 4 (purple), which is associated with the China lineage.

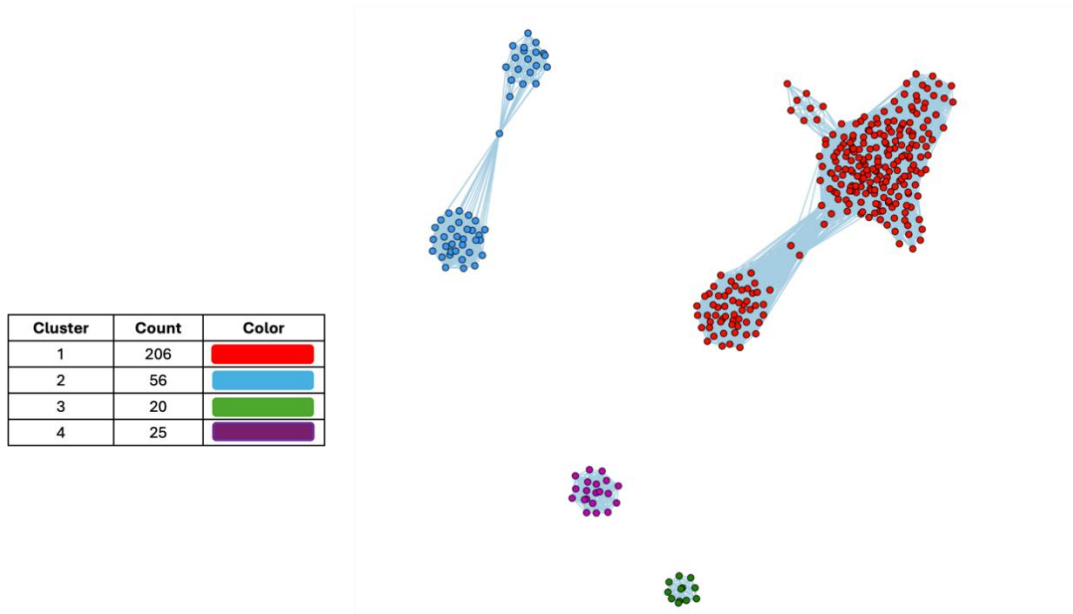


Figure 2. Molecular network analysis using MicrobeTrace identified four transmission clusters: Cluster 1 (red), Cluster 2 (blue), Cluster 3 (green), and Cluster 4 (purple).

The median network degree was 55, with a 75th percentile of 136, and these values were used to interpret network connectivity. Cluster 1 showed the highest connectivity, with many nodes exceeding the median and reaching or surpassing the 75th percentile and was mainly concentrated in northern and northeastern regions (Figure 3). Cluster 2 was detected across multiple regions

throughout the study period but exhibited degree values below the median. Cluster 3 was detected only during the early period (1998–2002), while Cluster 4 emerged during the later period (2011–2014) and was confined to border provinces. Spatial analysis indicated a shift in circulating clusters over time, from Clusters 1–3 in 1998–2002 to Clusters 1, 2, and 4 in 2011–2014 (Figure 4).

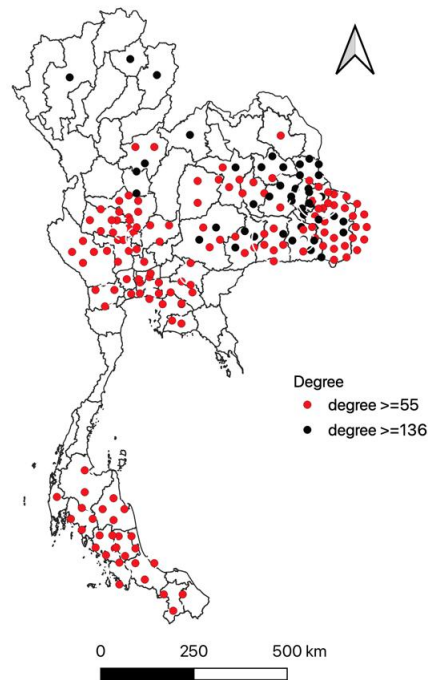


Figure 3. Geographic distribution of rabies virus isolates according to network degree.

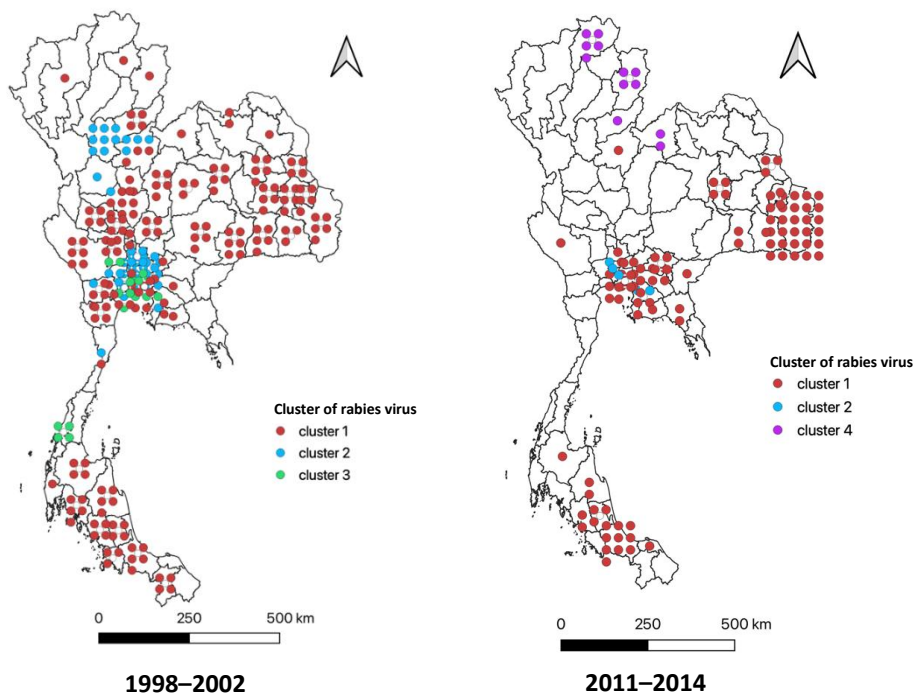


Figure 4. Spatial analysis showed a temporal shift in circulating clusters, from Clusters 1–3 in 1998–2002 to Clusters 1, 2, and 4 in 2011–2014.

Discussion

This study demonstrates the long-term circulation of multiple rabies virus lineages in Thailand and highlights the value of integrating phylogenetic, molecular network, and spatial analyses for rabies surveillance.^{11,18} The presence of multiple co-circulating clusters observed in this study is consistent with previous nationwide analyses, which identified two major phylogroups (TH1 and TH2) with distinct geographic distributions and evidence of virus movement associated with human-mediated dog translocation.⁷ The genetic distance threshold of 0.015 produced network structures that aligned well with phylogenetic clustering while maintaining epidemiological interpretability.¹⁷ This threshold was selected based on RNA virus evolutionary characteristics and supported by rabies molecular epidemiological studies, where nucleotide divergence of approximately 1–2% is considered appropriate for distinguishing epidemiologically related clusters. Accordingly, 0.015 (1.5%) was applied as a biologically meaningful and conservative cutoff consistent with observed genetic diversity and transmission patterns.

Cluster-specific patterns further illustrate transmission dynamics. Cluster 1 exhibited wide geographic distribution, persistence across both study periods, and high network connectivity, supporting previous evidence of endemic rabies maintenance in Thailand. These findings are consistent with sustained transmission within dog populations forming stable endemic cycles.^{10,19} The coexistence of multiple clusters with differing connectivity patterns reflects complex transmission dynamics, similar to previous reports of co-circulating clades in densely populated areas.⁸

Cluster 2 was detected throughout the study period across multiple regions but exhibited lower network connectivity, suggesting more localized or fragmented transmission, potentially influenced by vaccination coverage or localized control measures. Cluster 3 was detected only during the early study period, indicating limited persistence. However, this pattern may also reflect sampling bias associated with passive surveillance systems, as well as the impact of control measures such as increased vaccination coverage and dog population management.

Cluster 4 was identified in border provinces during the later study period and showed a strong genetic association with the China clade, supported by high bootstrap values (100%). Similar findings have been reported in Thailand, where China-associated rabies virus lineages were identified in northern and northeastern border areas.⁹ While this pattern suggests possible cross-border connectivity, similar observations have been reported in regional studies indicating that rabies virus lineages are shared across Southeast Asia due to historical viral exchange rather than recent introduction events.²⁰ Nevertheless, in the absence of contemporaneous

sequences from neighboring countries, recent cross-border transmission cannot be definitively confirmed. Therefore, this finding should be interpreted with caution as indicative of regional connectivity rather than definitive evidence of cross-border introduction.

The main contribution of this study lies in integrating molecular network analysis with phylogenetic and spatial approaches at a national scale. Network metrics, such as degree distribution, provide additional insight into transmission intensity and connectivity, complementing traditional phylogenetic analysis.

Although four genetic clusters were identified, all viruses belonged to the Asian group and classical rabies virus lineage, indicating continued effectiveness of existing vaccines.^{2,18} From a control perspective, mass dog vaccination and population management remain essential strategies. Previous studies have shown that at least 70% vaccination coverage in dog populations is required to interrupt transmission.⁸ In Thailand, national rabies control programs include vaccination campaigns, stray dog management, and public awareness initiatives. The observed heterogeneity in transmission patterns suggests that targeted interventions may be needed in high-connectivity areas and border regions.

From a One Health perspective, rabies control requires coordinated efforts across veterinary, public health, and environmental sectors, including local administrative organizations. The regional connectivity of rabies virus lineages further highlights the importance of cross-border collaboration and integrated surveillance systems.⁴

Although this study is based on historical data, it provides important baseline information on long-term transmission dynamics and can support future molecular surveillance and guide rabies elimination strategies.

Limitations

This study was limited to data from 1998–2002 and 2011–2014 due to the availability of complete metadata, and therefore may not fully represent current transmission dynamics. Reliance on passive surveillance and publicly available sequences may have introduced sampling and geographic biases, and the absence of contemporaneous sequences from neighboring countries limits confirmation of cross-border transmission.²¹ Despite these limitations, the findings provide valuable baseline information for future molecular surveillance efforts.

Public Health Recommendations

Rabies surveillance should be strengthened in areas with persistent circulation and high network connectivity, particularly in border provinces. Integrating molecular epidemiological tools into routine surveillance can improve

detection of transmission patterns. Sustained mass dog vaccination ($\geq 70\%$ coverage), targeted interventions in high-risk areas, and strengthened One Health collaboration, including cross-border coordination, are essential for effective rabies control.

Conclusion

Four distinct rabies virus genetic clusters circulated in Thailand between 1998 and 2014, showing differences in temporal occurrence, spatial distribution, and network connectivity. Cluster 1 exhibited widespread and persistent circulation with high connectivity, while Cluster 2 circulated throughout the study period with lower connectivity. Cluster 3 was confined to the early period, and Cluster 4 emerged later in geographically restricted border areas. Integrated phylogenetic, molecular network, and spatial analyses provided a comprehensive description of rabies virus circulation patterns in Thailand and support their application in rabies surveillance and control.

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Author Contributions

Bopit Puyati: Conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft, writing—review & editing. **Nipan Rongkhakulpipat:** Data curation, Resources, validation, writing—review & editing. All authors have read and approved the final manuscript.

Ethical Approval

This study used publicly available rabies virus nucleotide sequences obtained from GenBank and previously published surveillance studies. No human participants or live animals were directly involved in this research. Therefore, ethical approval and informed consent were not required.

Informed Consent

Not applicable. This study utilized retrospective surveillance data and did not involve human participants.

Data Availability

All rabies virus sequences analyzed in this study were obtained from publicly accessible databases (GenBank) and from previously published studies, with permission granted by the corresponding authors.

Conflicts of Interest

The authors are employed by the Department of Livestock Development, Thailand, which is responsible for rabies surveillance and control. However, the authors declare that we have no financial or commercial conflicts of interest related to this study.

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process

This manuscript was prepared with assistance from AI (artificial intelligence) tools for language editing and structure improvement. Scientific content and interpretation were verified by the authors.

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