



World Organisation  
for Animal Health  
Founded as OIE



# Molecular Techniques Supporting Rabies Prevention & Control

**Shrikrishna Isloor**

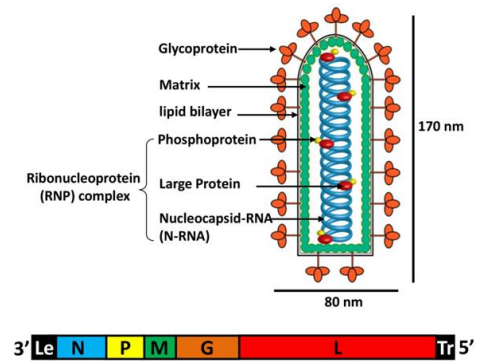
**Lab. Director, KVAFSU-CVA Rabies Diagnostic Laboratory**

**WOAH (Founded as OIE) Reference Laboratory for Rabies**

**Prof. & Head, Dept. of Vety. Microbiology, Veterinary College, KVAFSU,  
Hebbal, Bengaluru, INDIA**

## Molecular studies

- More rapid response plans for new outbreaks
- Real-time surveillance tools to guide rabies elimination initiatives.
- Revolutionized the diagnosis and surveillance of rabies.
- Significant in the AM detection of rabies (Sujith et al., 2017)
- Various RT-PCRs for AM & PM rabies diagnosis.
- Most of PCR versions target the N gene , followed by G (Mani and Madhusudhana, 2013).



**RABV evolution measurable on timescales similar to outbreaks in previously rabies-free areas or shifts to novel host species**

# Changing epidemiology of rabies in animals in South Asia

Different species of animals involved other than dogs

South Asia  
Dog – major vector



## India

Cat, Wolf, Jackal, Lion, Elephant, Horse, Deer, Livestock

## Nepal

Jackal, cat

## Bangladesh

Cattle

## Sri Lanka

rising cases of rabies in wild animals - Jackals and in areas at the village-forest interface; Cat, Deer, Livestock

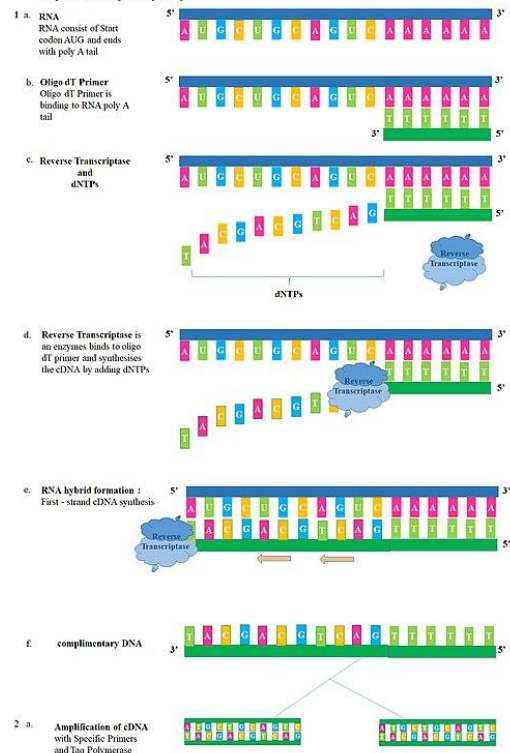


# Reverse Transcription-PCR (RT-PCR) / quantitative Real time –PCR (qRT-PCR)

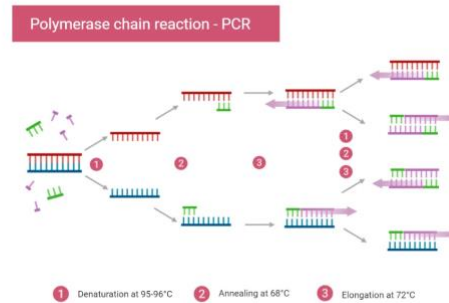
- **Purpose:** Rapid field confirmation and diagnostic surveillance
- **Applications:**
  - LN34 real-time RT-PCR (field use) → Rapid confirmation of RABV in animal samples; reduced response time (Demetria et al, 2023, Japan)
  - Country-specific N-gene RT-qPCR assays (Manalo et al., 2024 Philippines) → Increased national diagnostic sensitivity

## 4.8 Reverse transcription polymerase chain reaction (RT-PCR)

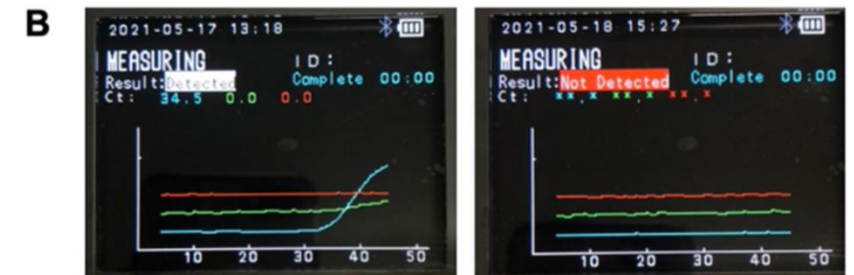
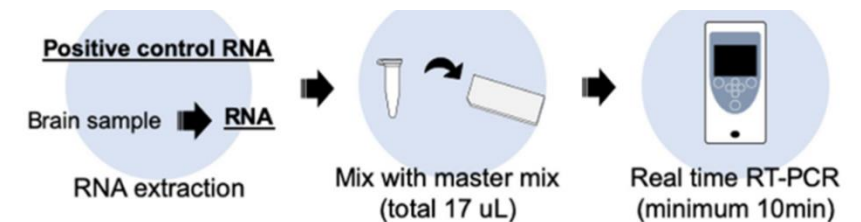
In RT-PCR, the RNA population is converted to cDNA by reverse transcription (RT), and then the cDNA is amplified by the polymerase chain reaction. The cDNA amplification step provides opportunities to further study the original RNA species, even when they are limited in amount or expressed in low abundance. Common applications of RT-PCR include detection of expressed genes, examination of transcript variants, and generation of cDNA templates for cloning and sequencing.



## Real Time PCR / quantitative PCR (qPCR)



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**Fig. 1** Overview of the PCR1100 assay. **A** Schematic illustration of the workflow of the real-time RT-PCR procedure used in this study. **B** Once the PCR reaction is complete, the amplification curve and results are displayed on the monitor. The left panel shows a positive result, whereas the right panel shows a negative result. Blue, green, and red lines indicate FAM, VIC, and Cy5 fluorescence, respectively



RESEARCH ARTICLE

# Multi-site evaluation of the LN34 pan-lyssavirus real-time RT-PCR assay for post-mortem rabies diagnostics

Crystal M. Gigante<sup>1</sup>, Lisa Dettinger<sup>2</sup>, James W. Powell<sup>3</sup>, Melanie Seiders<sup>2</sup>, Rene Edgar Condori Condori<sup>1</sup>, Richard Griesser<sup>3</sup>, Kenneth Okogi<sup>4</sup>, Maria Carlos<sup>4</sup>, Kendra Pesko<sup>5</sup>, Mike Breckenridge<sup>5</sup>, Edson Michael M. Simon<sup>6</sup>, Maria Yna Joyce V. Chu<sup>6</sup>, April D. Davis<sup>7</sup>, Scott J. Brunt<sup>7</sup>, Lillian Orciari<sup>1</sup>, Pamela Yager<sup>1</sup>, William C. Carson<sup>1</sup>, Claire Hartloge<sup>1</sup>, Jeremiah T. Saliki<sup>8</sup>, Susan Sanchez<sup>8</sup>, Mojgan Deldari<sup>9</sup>, Kristina Hsieh<sup>9</sup>, Ashutosh Wadhwa<sup>1</sup>, Kimberly Wilkins<sup>1</sup>, Veronica Yung Peredo<sup>10</sup>, Patricia Rabideau<sup>11</sup>, Nina Gruhn<sup>11</sup>, Rolain Cadet<sup>12</sup>, Shrikrishna Isloor<sup>13</sup>, Sujith S. Nath<sup>13</sup>, Tomy Joseph<sup>14</sup>, Jinxin Gao<sup>1</sup>, Ryan Wallace<sup>1</sup>, Mary Reynolds<sup>1</sup>, Victoria A. Olson<sup>1</sup>, Yu Li<sup>1\*</sup>



**Table 2. List of the laboratories that participated in the LN34 assay evaluation and the country or region of sample origin.**

Laboratory	Laboratory Location	Sample Origin
Maryland Department of Health and Mental Hygiene	United States	United States
Pennsylvania Department of Health	United States	United States
New Mexico Department of Health	United States	United States
California Department of Public Health	United States	United States
Wisconsin State Laboratory of Hygiene	United States	United States
Wadsworth Center, New York State Department of Health	United States	United States
University of Georgia	United States	United States
United States Army Public Health Command Europe	Germany	Middle East, Europe
Animal Health Centre, British Columbia	Canada	Canada
Research Institute for Tropical Medicine	Philippines	Philippines
Ministère de l'Agriculture	Haiti	Haiti
Instituto de Salud Pública de Chile	Chile	Chile
Karnataka Veterinary, Animal and Fisheries Sciences University	India	India
Centers for Disease Control and Prevention	United States	Georgia
		Asia
		Ethiopia

<https://doi.org/10.1371/journal.pone.0197074.t002>

**A multi-site evaluation of the LN34 assay in 14 laboratories.** A total of 2,978 samples (1,049 DFA positive) from the Americas, Europe, Africa and Asia were tested.

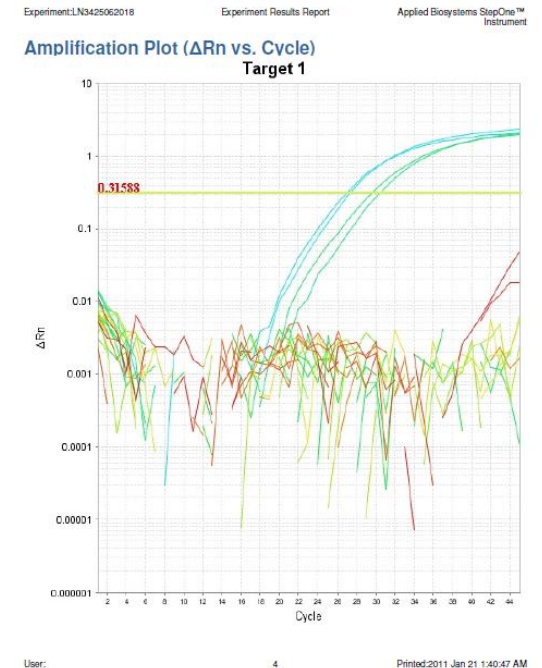
**High sensitivity (99.90%), specificity (99.68%)**

**Detects viral RNA in fresh, frozen, archived, deteriorated and formalin-fixed brain tissue.**

(Gigante et al., 2018)

**Positive (CT <35) control**

**All negative for Rabies (>45 CT)**



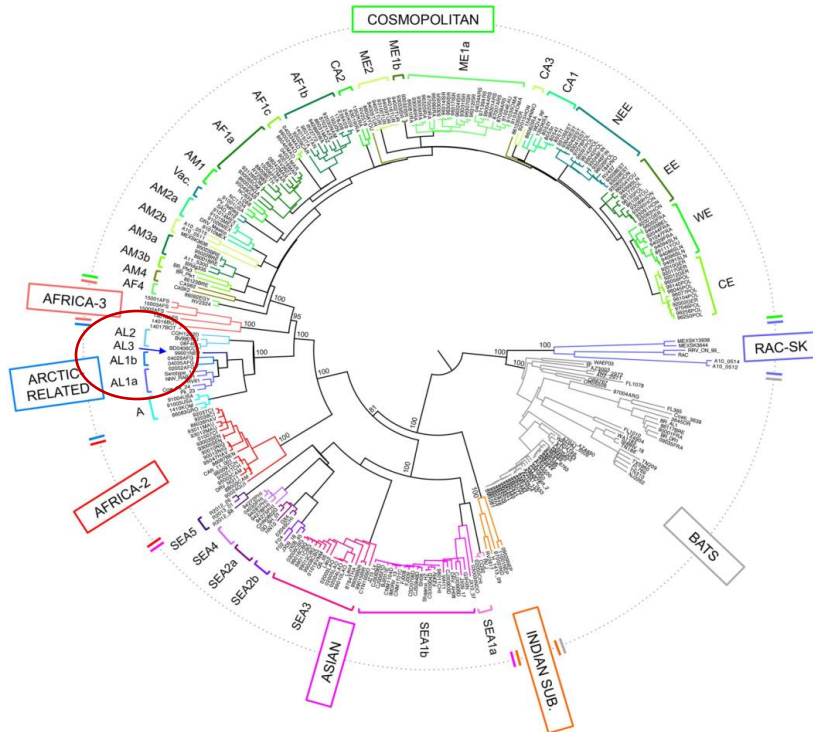
# Sanger Sequencing and Phylogeny

**Purpose:** Targeted sequencing to assign lineages

**Applications:**

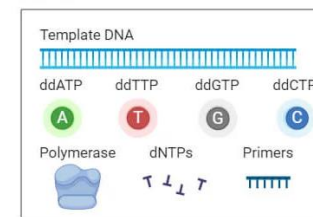
N/G gene sequencing → Phylogenetic confirmation of dog-mediated lineages (Isloor et al., 2014, Wolf; Chupin et al., 2023, Russia)

Outbreak tracing in low-resource settings where WGS unavailable

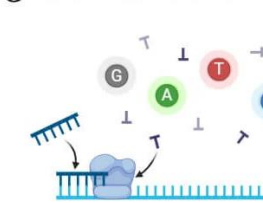


## Sanger Sequencing

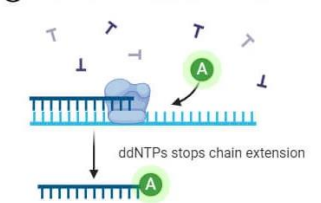
### Reagents



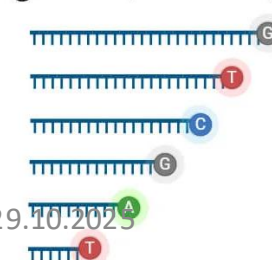
### ① Primer annealing and chain extension



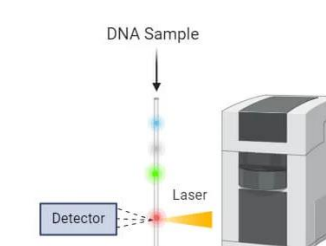
### ② ddNTP binding and chain termination



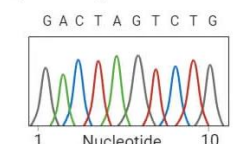
### ③ Fluorescently labelled DNA sample



### ④ Capillary gel electrophoresis and fluorescence detection

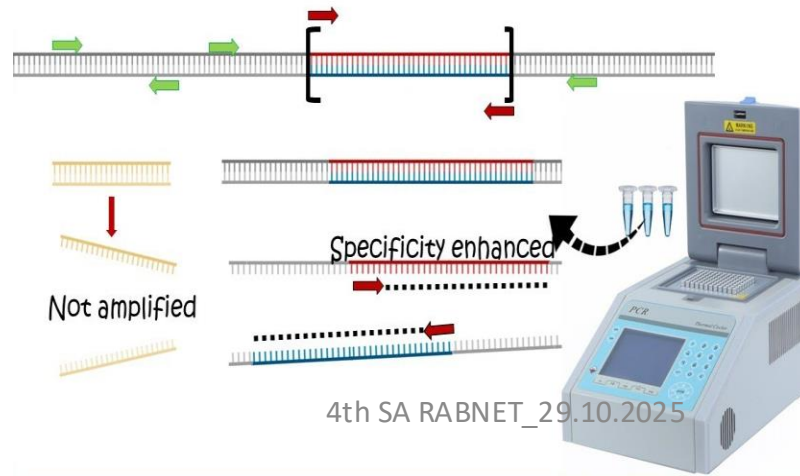


### ⑤ Sequence analysis and reconstruction

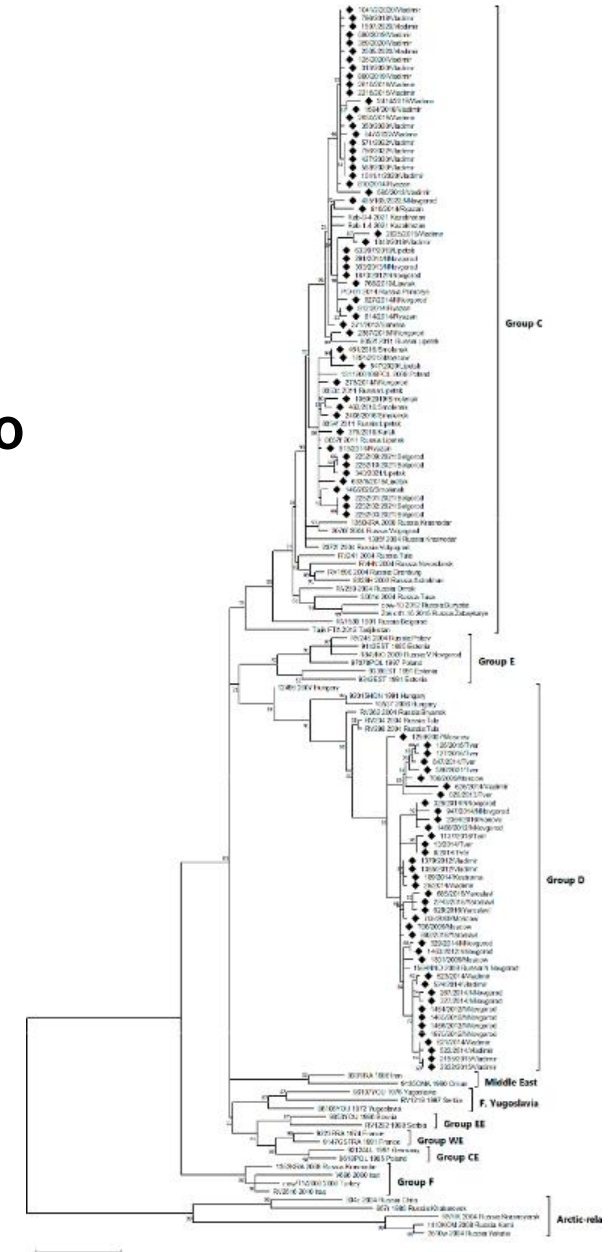


# Conventional / Nested PCR

- **Purpose:** Gene-targeted amplification for genotyping & surveillance
- **Applications:**
  - N-gene PCR + Sanger sequencing → **Clade identification** for outbreak tracing (Chupin et., 2023, Russia)
  - Pan-lyssavirus nested PCR → Detection of non-canine reservoirs in wildlife (ANSES, 2022)



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## Review Article

# First Case Report of Rabies in a Wolf (*Canis Lupus Pallipes*) from India

Isloor S<sup>1</sup>, Marissen WE<sup>2</sup>, Veeresh BH<sup>1</sup>, NithinPrabhu K<sup>1</sup>, Kuzmin IV<sup>3</sup>, Rupprecht CE<sup>3,4\*</sup>, Satyanarayana ML<sup>1</sup>, Deepti B R<sup>1</sup>, Sharada R<sup>1</sup>, Neelufar MS<sup>1</sup>, Yathiraj S<sup>1</sup> and Abdul Rahman S<sup>1</sup>

## \*Corresponding author

Rupprecht CE, Global Alliance for Rabies control, Ross University School of Veterinary Medicine, USA, Email: CRupprecht@RossU.edu

Submitted: 08 July 2014

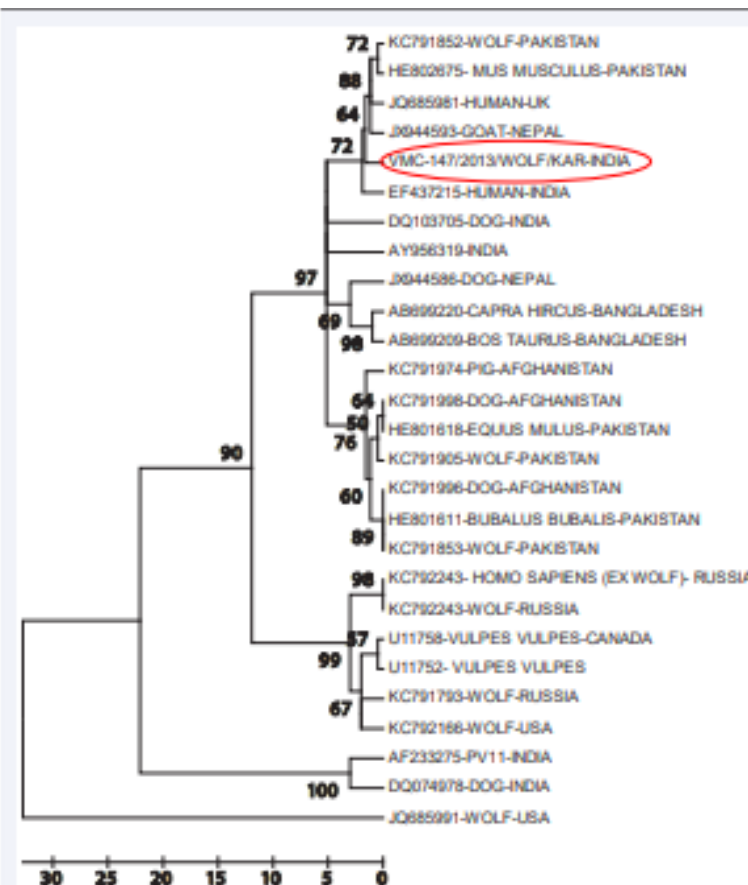
Accepted: 01 November 2014

Published: 06 November 2014

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**Figure 8** Phylogenetic tree based on the analysis of 524 amino acids in the complete G gene of wolf rabies virus.



# KVAFSU-CVA RDL STUDY ON COMPLETE N GENE of WILD CARNIVORES RABV

GC-1

KARNATAKA

ARCTIC 1a-like Lineage

MANIPUR, ASSAM and  
MAHARASHTRA

GC-2

ASIAN CLADE (SEA5 SUBCLADE)

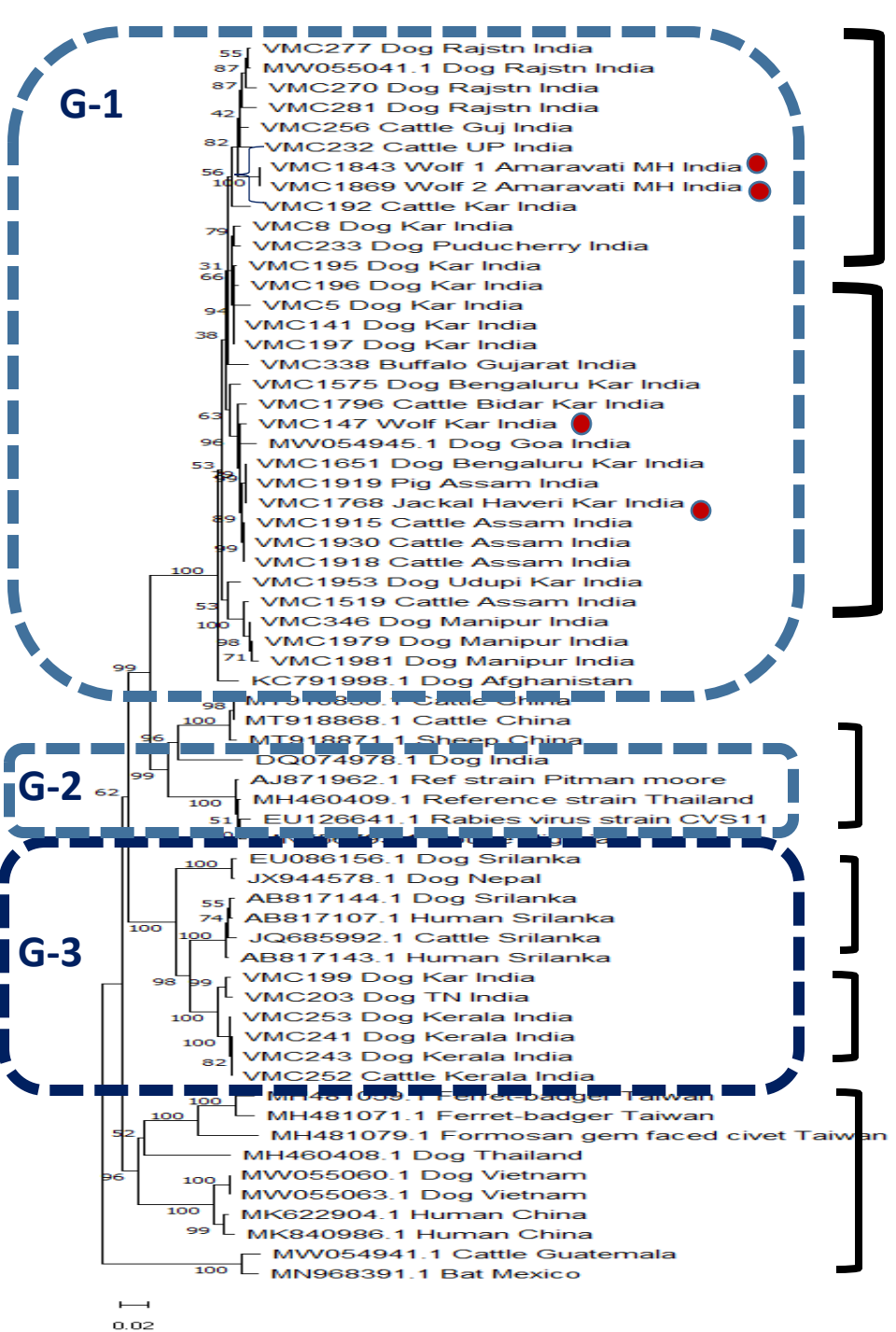
GC-3

SRI LANKAN

KERALA AND TAMIL NADU

INDIAN SUB-CONTINENT lineage

# KVAFSU-CVA RDL STUDY ON COMPLETE G GENE of WILD CARNIVORES RABV



MANIPUR, ASSAM ,  
MAHARASHTRA

KARNATAKA

ARCTIC 1a-like lineage

REF CVS11

SRI LANKA

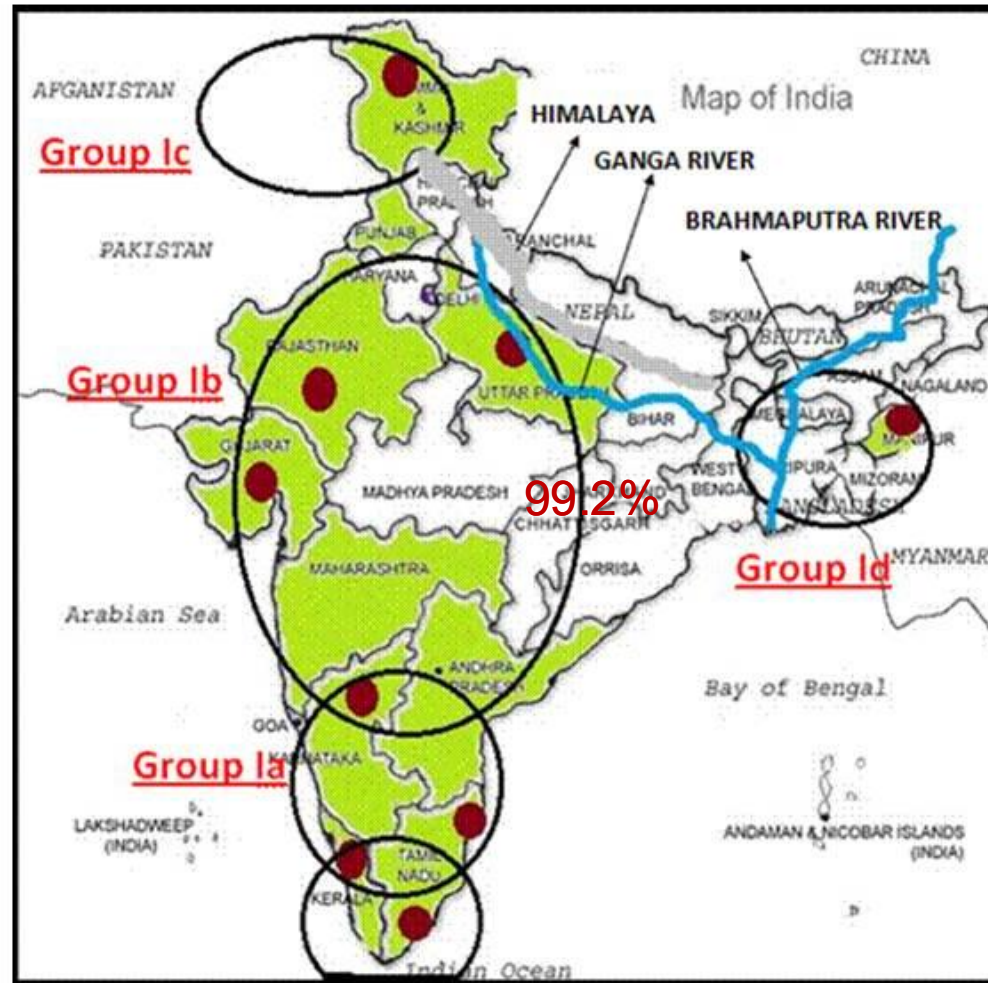
INDIAN-SUBCONTINENT lineage

KERALA AND TAMIL NADU

ASIAN CLADE (SEA5 SUBCLADE)



## G-L intergenic region based clustering of RABV from dogs and cattle in India



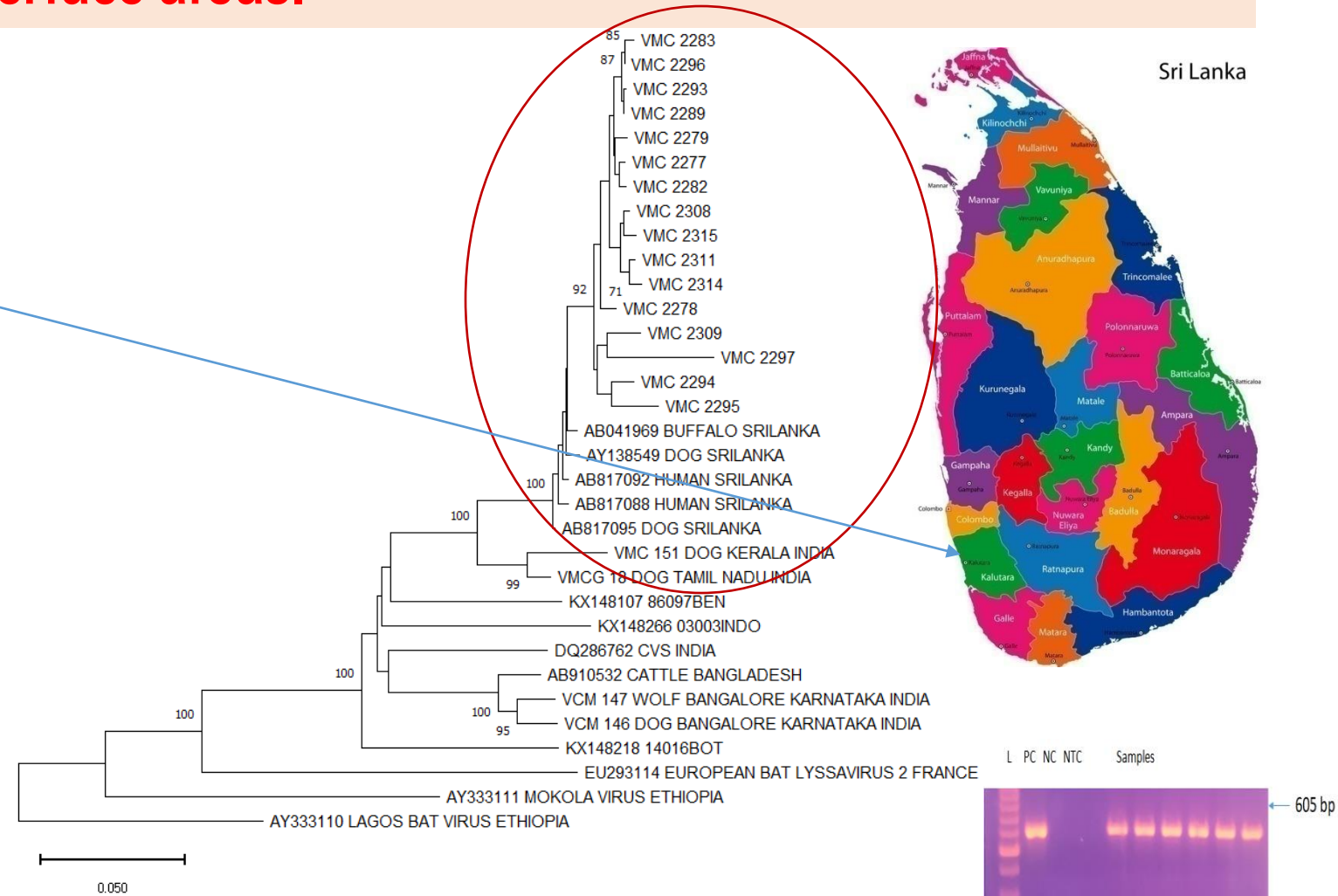
Bill & Milinda Gates sponsored Young Investigator award during 17<sup>th</sup> ICID International conference at Hyderabad, India in 2016



Recently, Sri Lanka has seen rising cases of rabies cases in wild animals such as jackals and in areas at the village-forest interface areas.



Samples list	No. of samples
Jackal	21
Cow	10
Dog	4
Goat	4
Cat	1
Deer	1
Total no.	41



Indian subcontinent lineage

# Phylogeography & Phylodynamics

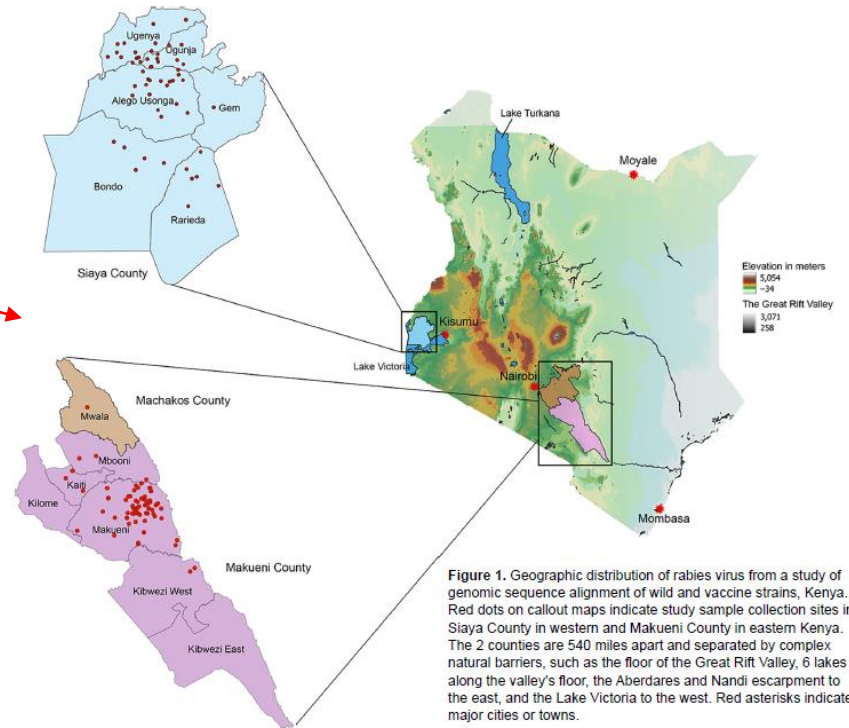
**Purpose:** Trace geographic spread & estimate lineage timing

## Applications:

National phylogeography (Philippines) → Movement corridors identified (PubMed, 2023)

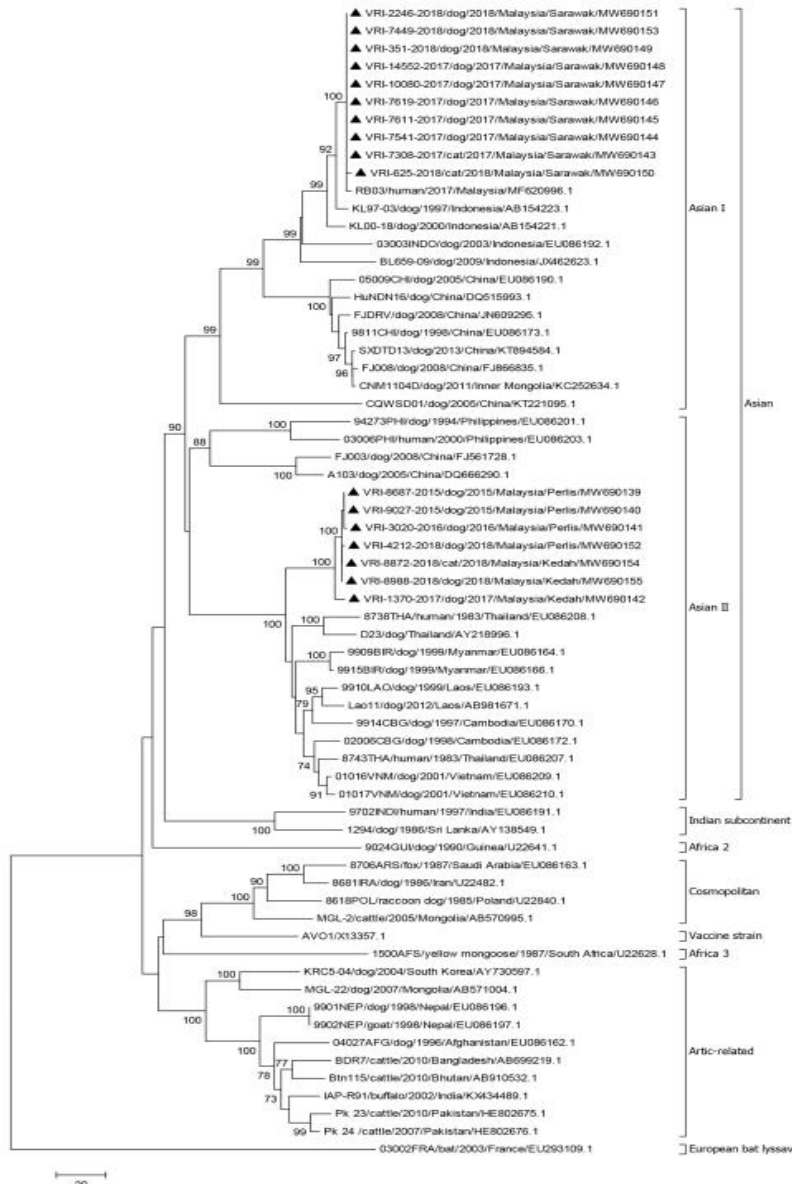
Molecular clock analysis → Timing of introductions & mutation rates in Kenya (Wambugu et al., 2024, Kenya)

Africa 1 a clade



Africa 1b clade

# Phylogenetic analysis of nucleoprotein gene of Rabies virus in Malaysia from 2015 to 2018 (Leow et al., 2021)



All Malaysia RABVs were grouped in Asian clade

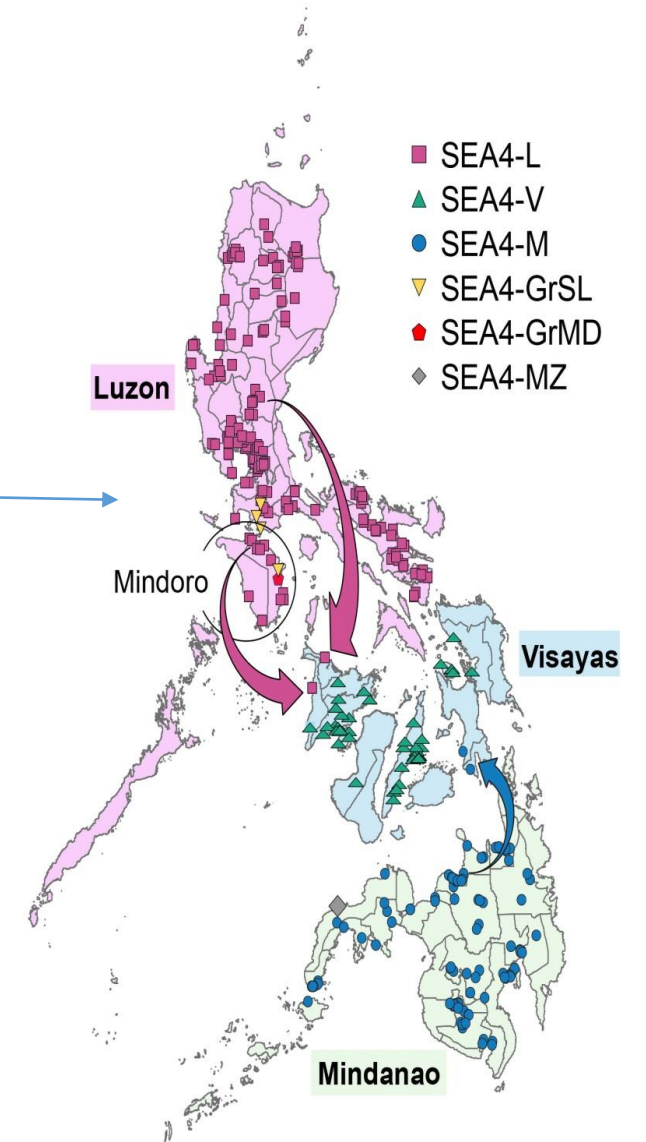


Asian I and Asian II of RABVs are circulating in the country from 2015 to 2018

RABVs from the rabies endemic bordering countries might have been introduced to Malaysia

# Source Attribution & Outbreak Investigation

- **Purpose:** Confirm human-animal linkages and outbreak origin
- **Applications:**
  - Human-dog case matching via sequencing (CDC, 2023)
  - WGS + epidemiological data integration → Reintroduction vs persistence clarified (Jhang et al., 2025, Mol. Bio. Evol, Phillipines)





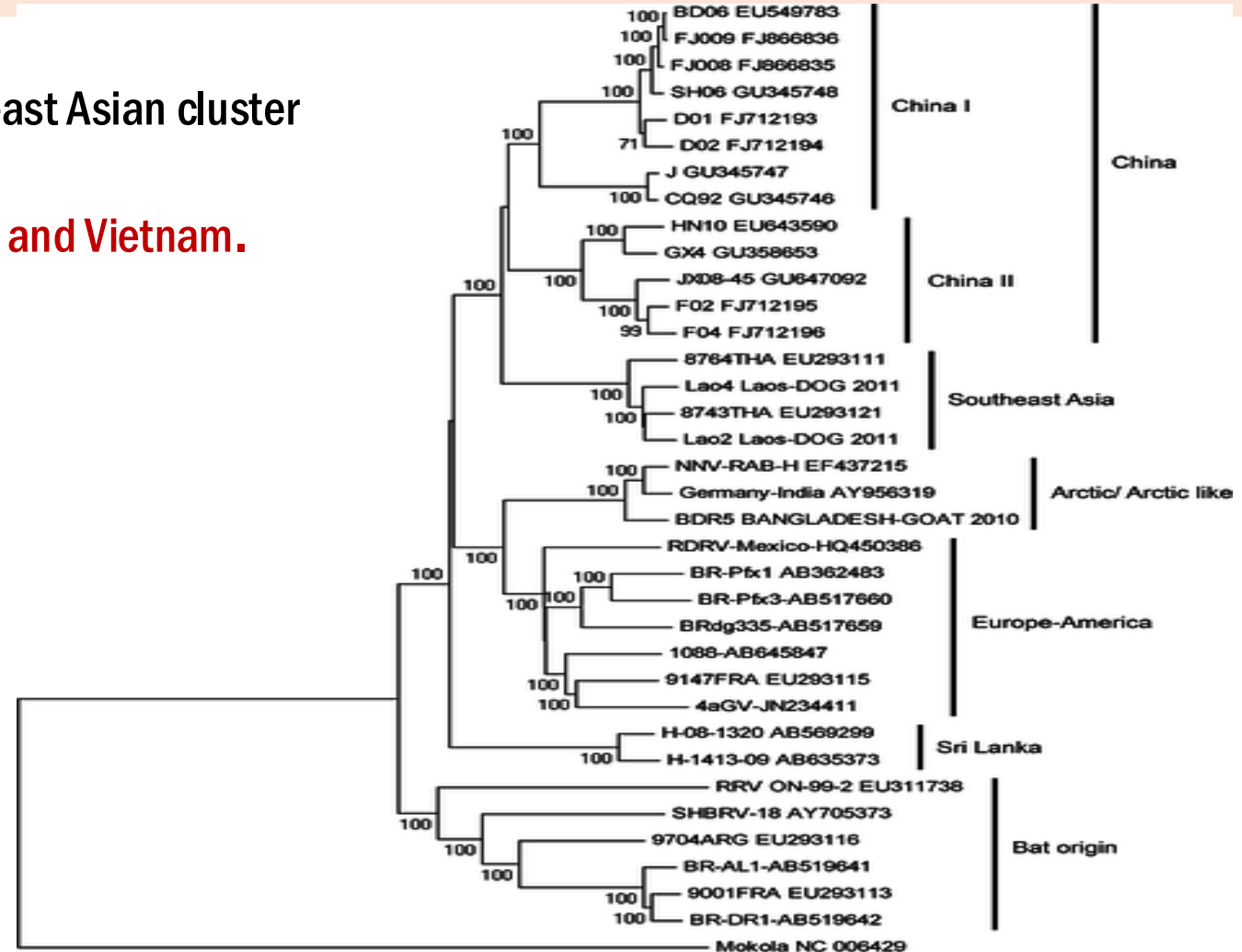
# Molecular Epidemiology of Rabies Viruses Circulating in Two Rabies Endemic Provinces of Laos, 2011–2012: Regional Diversity in Southeast Asia

(Ahmed et al., 2015)

- **Three rabies virus lineages** belonging to the Southeast Asian cluster are currently circulating in **Laos**
- Closely related to viruses from **Thailand, Cambodia and Vietnam**.



Lineages diverged from common ancestors as recently as 44.2 years and as much as 55.3 years ago-periodic virus invasions

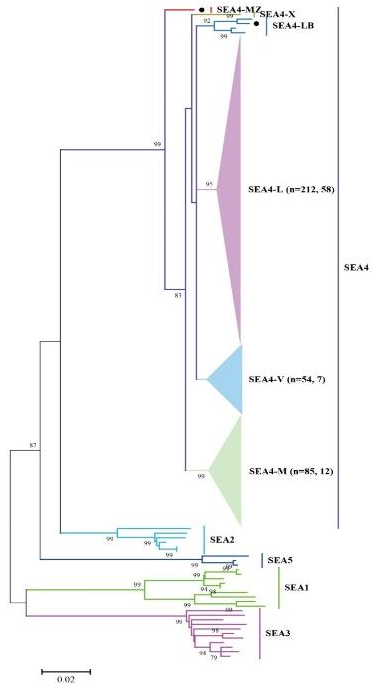


# Molecular Epidemiology of Rabies in the Philippines, 2023. A research project between CVRI, China and ADDRL, Philippines

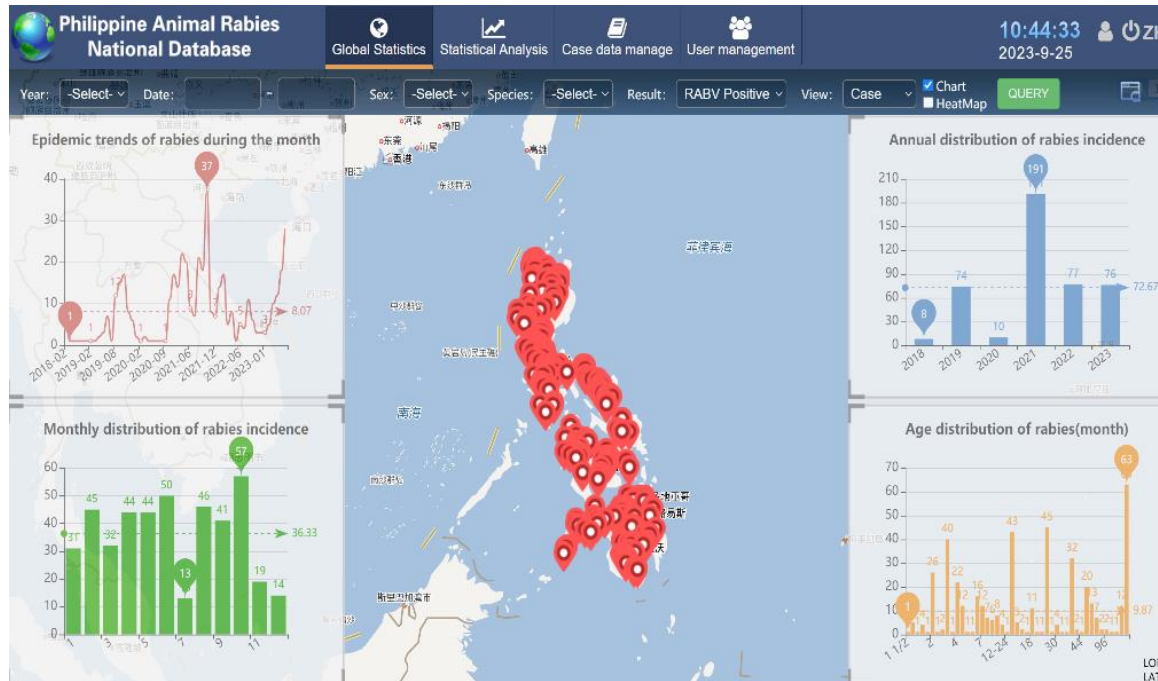
Key activity: 1. Understanding genetic diversity, distribution and transmission dynamics of RABV in Philippines

2. Establishing an animal rabies information system

Whole Genome Sequence based approach



Phylogenetic analysis of RABVs in the Philippines



Phylogenetic analysis of RABVs in the Philippines



Contents lists available at ScienceDirect

# Comparative Immunology, Microbiology and Infectious Diseases

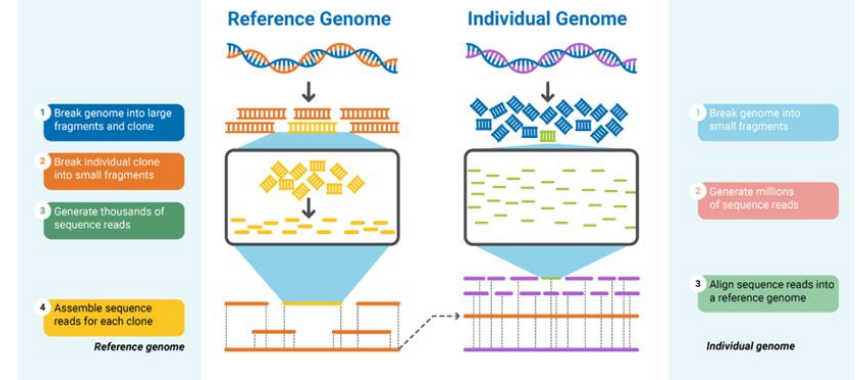
journal homepage: [www.elsevier.com/locate/cimid](http://www.elsevier.com/locate/cimid)

Full length article

## Integrating one health strategies for rabies control: Insights from Jackal-mediated human rabies in Northeast India

Jyoti B. Dutta<sup>a,1</sup>, MA Ashwini<sup>b,1</sup>, Ditul Barman<sup>c</sup>, Pranab Das<sup>d</sup>, Raktim Tamuli<sup>e</sup>, K. Dhanya<sup>b</sup>,  
CN Varun<sup>b</sup>, Shubhangi Chandel<sup>b</sup>, P. Soundarya<sup>b</sup>, Shrikrishna Isloor<sup>f</sup>, Reeta S. Mani<sup>b,\*</sup>

## WHOLE GENOME SEQUENCING



J.B. Dutta et al.

Comparative Immunology, Microbiology and Infectious Diseases 116 (2025) 102287

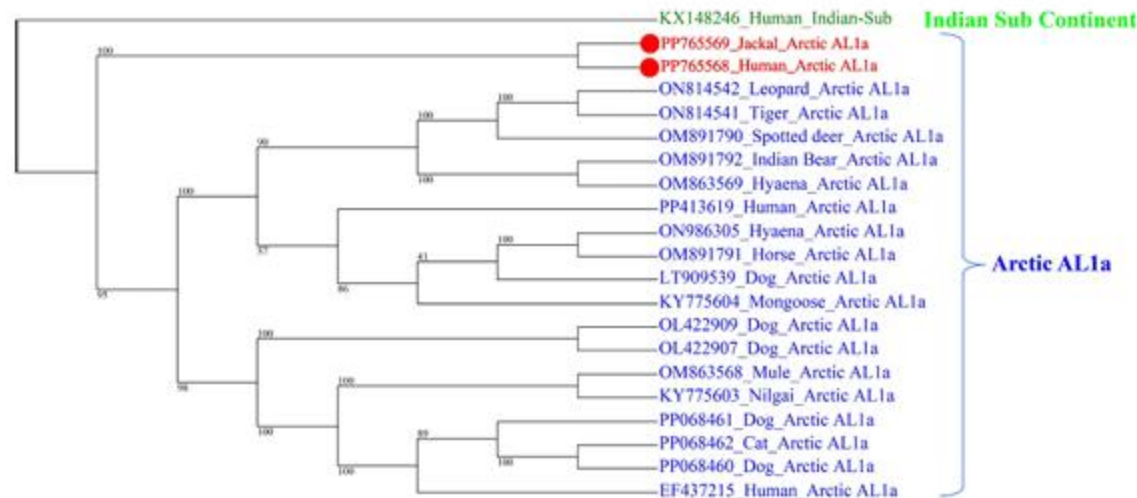


Fig. 1. Legend: Phylogenetic tree indicates relatedness between the jackal and human rabies virus sequences from the study (in red color).

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# Emerging Techniques

## Metagenomic NGS – improves sensitivity

Study of all the genetic material found in a mixed community of microbes directly from their environment,

Wildlife metagenomics → Discovered divergent lyssaviruses (Lin et al., 2025, China)

## SNP-Based Typing & Lineage Tools

Rapid lineage typing (Kumar et al., 2024)

## RABV-GLUE

Genes Linked by Underlying Evolution- A Bioinformatics platform

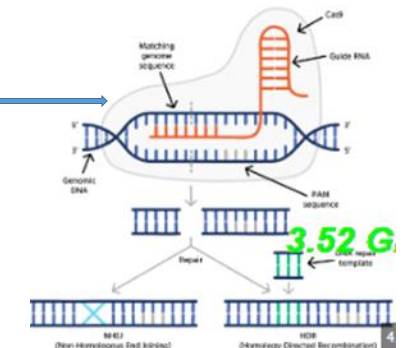
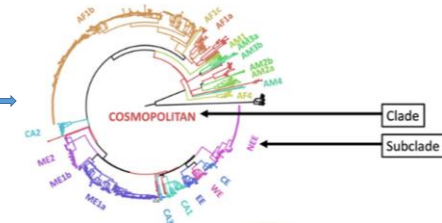
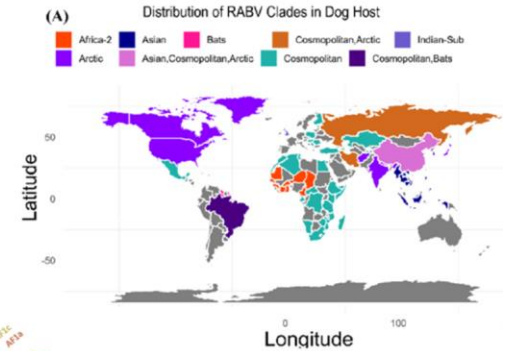
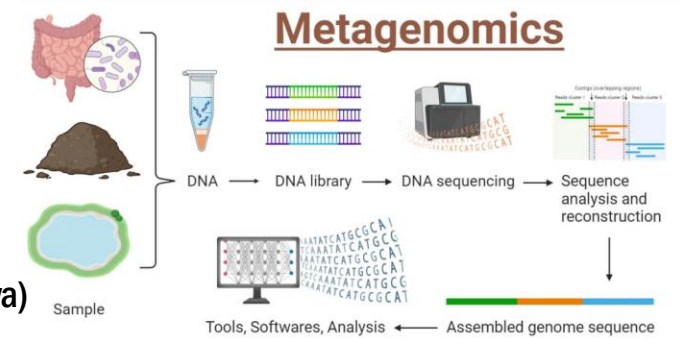
## CRISPR-based Detection (SHERLOCK / DETECTR)

Portable diagnostic for low-resource settings

## Machine Learning (ML)

AI that allows computers to learn from experience or data

(Liu et al., 2023, China: CSF, Saliva)





## Summary

Technique	Core Application	Key Impact	Reference
RT-PCR (KVAFSU-CVA RDL)	Field confirmation	Rapid case detection	BioMed Central, 2023
WGS	Outbreak genomics	Source tracking	PubMed, 2024
Metagenomics	Novel virus detection	Early warning	Frontiers, 2023
Phylogeography (KVAFSU-CVA RDL)	Spread mapping	Targeted vaccination	PubMed, 2023
CRISPR	Emerging Dx	Field readiness	RSC, 2024

## Key Takeaways

- Enables **faster diagnosis, lineage tracing, and targeted interventions.**
- Genomic epidemiology now central to **One Health rabies elimination strategies.**
- Emerging CRISPR and ML methods promise **next-generation surveillance.**

## **WOAH RL can support SAARC countries in enhancing their molecular diagnostic capacity and setting up Lab.**

- **Molecular epidemiology, if brain samples submitted**
- **Virtual training programme on Molecular Techniques**
- **Hands on experience on Molecular Techniques**
- **Need based visit to support setting up lab.**