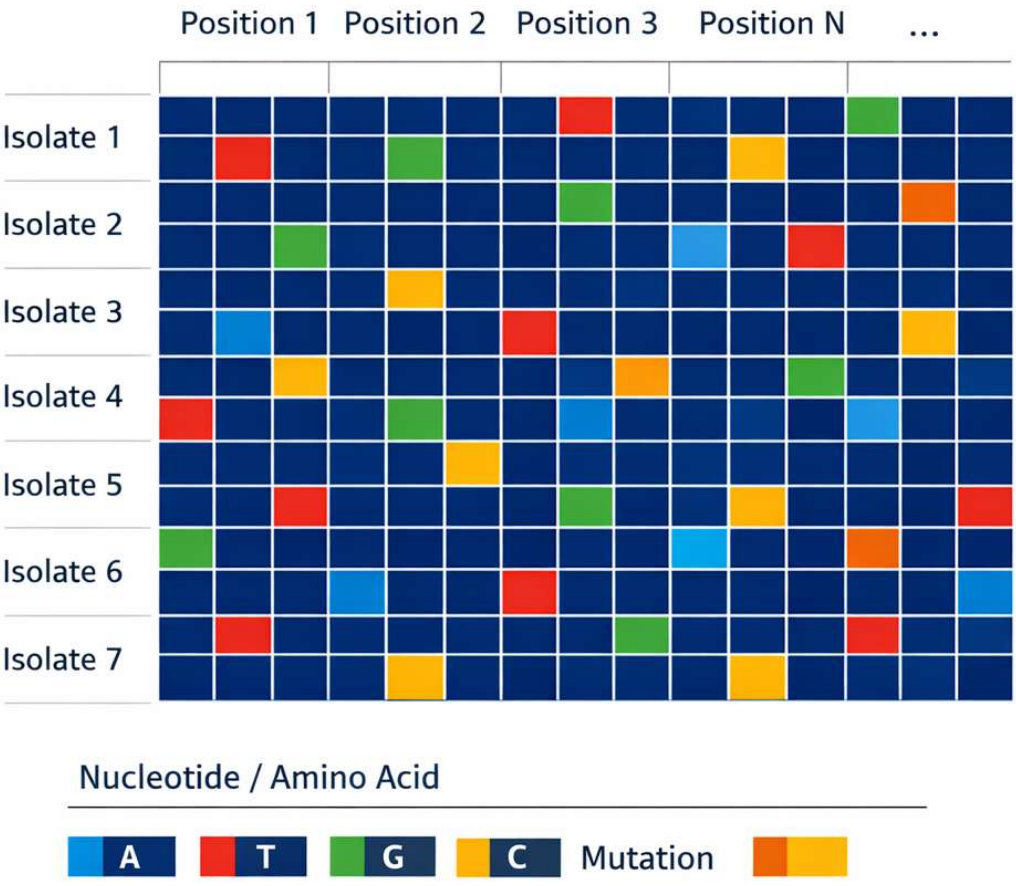
Genetic Diversity and Evolutionary Dynamics of Kyasanur Forest Disease Virus in India:

A One Health Perspective



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Abbreviations

AHFV	Alkhurma Hemorrhagic Fever Virus
C	Capsid Protein
E	Envelope Protein
ICD-10	International Classification of Diseases, 10th Revision
ICMR	Indian Council of Medical Research
kb	Kilobase
km	Kilometre
KFD	Kyasanur Forest Disease
KFDV	Kyasanur Forest Disease Virus
NIV	National Institute of Virology
NS	Non-Structural Protein
NS1–NS5	Non-Structural Proteins 1 to 5
NS2A / NS2B	Non-Structural Protein 2A / 2B
NS3	Viral Protease and Helicase
NS4A / NS4B	Replication-associated Membrane Proteins
NS5	RNA-Dependent RNA Polymerase
nt	Nucleotide(s)
prM/ M	Premembrane / Membrane Protein
RNA	Ribonucleic Acid
~	Approximately (used to denote estimated values)

Executive Summary

Kyasanur Forest Disease (KFD) is a tick-borne zoonotic viral disease of major public health importance in India. Depending on the tick stage (larvae, nymphs, adults), the virus can be transmitted to different host types including various wildlife and livestock species, and humans. Humans and some monkey species develop visible symptoms while other animal species seem to be asymptomatic carriers. Therefore, the presence of dead monkeys that test positive for the KFD virus (KFDV) signal viral activity in the region; these monkeys act as important sentinel species and data regarding monkey death can contribute to an early warning system.

First identified in 1957 in Karnataka, the disease has gradually expanded across the Western Ghats to Kerala, Tamil Nadu, Goa, and Maharashtra. This continued geographic spread and recurring seasonal outbreaks highlight the need to better understand the virus's ecology, evolution, and transmission pathways.

The KFDV, a member of the Flaviviridae family, has a positive-sense Ribonucleic Acid (RNA) genome (~10.7–11 kilobase (kb)) encoding structural and non-structural proteins required for replication and host interaction. Despite nearly seven decades of circulation, genomic studies show remarkably low genetic diversity, with only ~2.24% nucleotide (nt) variation among isolates from 1957–2020. This strong genetic conservation indicates long-term persistence in sylvatic (forest-related) cycles rather than rapid evolution through human transmission.

Recent analyses reveal gradual diversification into region-specific subgroups, reflecting localized ecological adaptation of KFDV. The virus evolves slowly (~10^{-4} substitutions per site per year) and spatial models indicate that it spreads at an estimated rate of about 60 km per year, likely driven by movement of wildlife hosts which might carry the virus inside their body or infected ticks on their body.

Current evidence shows that genetic variation has not significantly altered disease symptoms, which remain characterized by a febrile illness with occasional haemorrhagic or neurological complications. Although certain mutations may influence viral fitness or immune interactions, no clear association with increased severity or recent geographic emergence has been established. The detection of cases in new areas is likely due to improved surveillance and diagnostics rather than major viral change.

Important knowledge gaps persist, including limited long-term genomic data, insufficient understanding of wildlife and tick-mediated spread, inadequate integration of ecological and molecular studies, and fragmented surveillance across sectors.

Future efforts must move beyond isolated research towards integrated One Health surveillance. Investments in integrated genomic, ecological and epidemiological surveillance can provide information regarding disease spread, support evidence-based public health interventions and early warning systems and strengthen India's preparedness for emerging zoonotic diseases.

1. Background

Kyasanur Forest Disease (KFD) an important wildlife-linked tick-borne zoonotic disease found in India. Since its discovery in Karnataka in 1957, the disease has gradually expanded across the Western Ghats, affecting mainly forest-dependent communities and also certain monkey species (*Macaca radiata* and *Semnopithecus entellus*). The continued appearance of cases in new areas highlights the need to better understand how the virus persists, spreads and responds to environmental change (Shah et al., 2018).

Studying the genetic diversity of zoonotic and vector-borne pathogens like KFDV is essential for understanding how these evolve, spread, and adapt to different hosts, environments, and control measures. Genetic analysis helps identify emerging variants, trace transmission pathways across human, animal, and vector populations, and detect changes that may influence virulence, host range, or resistance to diagnostics, vaccines, or treatments (Kaushal et al., 2025).

Such knowledge strengthens surveillance systems, enables early warning of outbreaks, and supports the design of targeted public health interventions, ultimately improving preparedness and response through a One Health approach that integrates human, animal, and environmental health.

This document consolidates current scientific evidence on the genetic variation of KFDV to better understand its evolutionary dynamics, geographical spread, and the potential implications of viral diversity for disease patterns.

2. Epidemiology of KFDV in India

KFDV circulates among multiple hosts such as small mammals, monkeys, large mammals and humans. However, monkeys and humans are most susceptible to KFDV and develop clinical symptoms. Transmission to humans occurs primarily through bites of infected hard ticks belonging to the genus *Haemaphysalis* (Shah et al., 2018). Most patients (up to 80°%) recover without major complications. Approximately 20°% develop a biphasic illness, and a small proportion progresses to severe haemorrhagic or neurological manifestations.

KFDV was first identified in 1957 in the forest regions of Shivamogga District, Karnataka, India. Until 1971, KFDV was endemic to the Sagar, Sorab, and Shikaripur taluks of Shivamogga District. By 1972, KFD cases were reported in Sirsi Taluk, Uttara Kannada District. To date, KFD cases have been reported from multiple districts of Karnataka.

Outside Karnataka, KFDV was detected in monkeys in Nilgiris, Tamil Nadu, in 2012, the first confirmed occurrence beyond Karnataka. Subsequently, human cases were reported from Wayanad and Malappuram districts of Kerala (2013–2014), followed by outbreaks in northeastern Goa (2015) and Maharashtra (2016). The geographic expansion of KFD demonstrates that disease risks do not respect administrative boundaries. Effective management therefore requires coordination among health, forest, veterinary and local government institutions across multiple States. In addition, there is a need to better understand viral evolution and spatio-temporal transmission dynamics (Chakraborty et al., 2019).

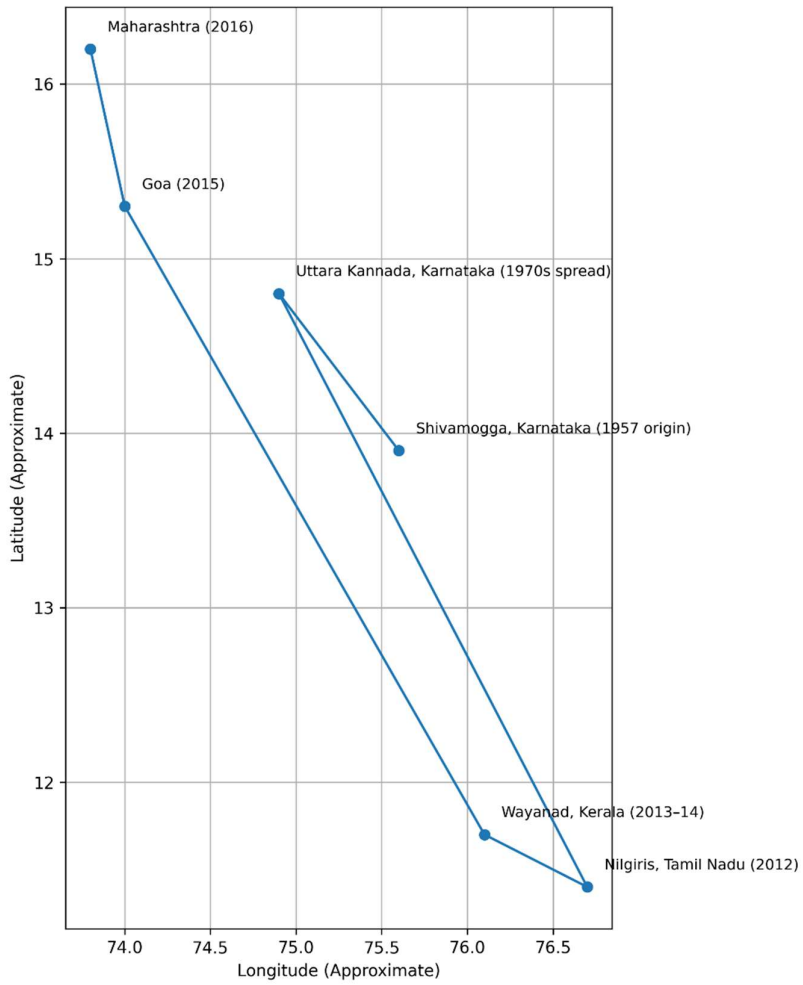

Figure1: Schematic spread of KFD in India across Western Ghats (based on first reported cases)

3. The structure of KFDV

KFDV is an enveloped, positive-sense, single-stranded RNA virus with a genome of approximately 10,774 to 11,000 nt (10.7–11 kb) in length (Bohra et al., 2025). The genome encodes a single polyprotein that is cleaved into:

- Structural proteins: Capsid (C), Premembrane/ Membrane (prM/M), and Envelope (E)
- Non-structural proteins (NS): NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

Numerous isolates collected between 1957 and 1972 are preserved at the Indian Council of Medical Research–National Institute of Virology (ICMR-NIV), Pune. Work with the virus was previously limited due to laboratory-acquired infections until appropriate high-containment facilities were established in 2004 (Mehla et al., 2009).

The International Classification of Diseases, 10th Revision (ICD-10), 2017 categorizes KFD under code A98.2, which falls under other viral haemorrhagic fevers. According to International Biosafety rules, KFDV is a level 4 pathogen (Munivenkatappa et al., 2018).

Table 1: Functional characteristics of structural and non-structural proteins encoded by KFDV

Structural proteins (virion formation)	
C (Capsid)	Packages viral RNA and forms the nucleocapsid
prM/M (Premembrane/ Membrane)	Assists viral assembly and maturation
E (Envelope)	Critical for host-cell attachment, membrane fusion, and immune recognition (major antigenic target)
Non-structural proteins (replication & pathogenesis)	
NS1	Viral replication and immune modulation; secreted form used in diagnostics research
NS2A / NS2B:	Membrane-associated proteins; NS2B acts as a cofactor for NS3 protease
NS3	Helicase and protease functions – essential for polyprotein processing
NS4A / NS4B	Modifies host-cell membranes to create replication complexes
NS5	RNA-dependent RNA polymerase; responsible for genome replication and viral evolution

4. Genetic diversity of KFDV in India

KFDV was first isolated and identified from sick and dead monkeys in Shivamogga forests during 1957. Subsequent studies sequenced viral isolates from humans, monkeys, and ticks collected between 1957 and 2020 across Karnataka, Kerala, Tamil Nadu, Goa and Maharashtra using both whole-genome sequencing and envelope (E) gene analysis to understand the genetic diversity of the virus.

Overall, KFDV shows remarkably low genetic diversity despite circulation over several decades. A study from 2019 revealed an overall nucleotide divergence of 2.24°% and an amino acid divergence of 0.75°% among KFDV isolated from 1957 to 2017 across five KFD affected States. Interestingly, the level of divergence during the period between 1957 and 1972 was found to be even lower, i.e. 0.31°%/ 0.30°% at the nucleotide / amino acid level (Yadav et al., 2019). Another study, conducted in 2009 which includes 47 samples from 1957 to 1972 and 1 sample from 2006, also showed a low genetic diversity of KFDV of 1.2°%. However, this study induces only the prM, E and NS5 genes but, not the whole genome (Mehla et al., 2009).

Although such low genetic variability is unusual for vector-borne flaviviruses, a similar pattern (only approximately 1.1% nucleotide and 0.8% amino acid divergence) has been reported for another tick-borne flavivirus, Alkhurma hemorrhagic fever virus (AHFV), in human isolates collected over a 15-year period (Dodd et al., 2011). AHFV, reported from Saudi Arabia and Egypt, is closely related to KFDV. Both share about 97°% of their genes, although evolutionary evidence suggests the two diverged around 700 years ago, possibly linked to historical animal movement or migratory routes.

Collaborative investigations led primarily by the ICMR-NIV, Pune, have clarified the origin and relationships of several reported KFDV isolates worldwide. Detailed analysis of the 1989 Chinese “Nanjianyin virus” showed that it was not an independent strain but almost identical (99.92°%) to the 1957 Indian reference strain (P9605), indicating likely laboratory cross-contamination after global distribution of the reference virus (Mehla et al., 2009).

5. Evolutionary dynamics and pattern of dispersal of KFDV across Western Ghats

Although the KFDV remained largely genetically conserved between its initial identification in 1957 and the 1970s, recent sequencing and phylogenetic studies indicate the gradual emergence of distinct viral subgroups over time. Nevertheless, evidence suggests that earlier ancestral strains continue to circulate among animal hosts and vectors in endemic regions, indicating co-circulation of older and more recent viral lineages.

Since the 2000s, KFDV has diversified into four geographically associated subgroups, Karnataka, Goa, Maharashtra, and Tamil Nadu, with specific amino acid variations observed in the Capsid, prM, and non-structural proteins. These patterns suggest ongoing viral evolution and local ecological adaptation, though within a relatively narrow genetic range compared to many other flaviviruses.

Strains circulating between 2006 and 2017 differ from the early Karnataka isolates (1957-1972) by about 2.76°% across the genome. The greatest nucleotide divergence occurs in the Capsid (3.27°%), followed by the Envelope (3.22°%) and NS2 (2.91°%) genes. Comparisons of samples collected between 2006 and 2017 regarding the genetic divergence across hosts show a divergence between human and tick-derived isolates of 2.39°% while divergence between monkey and tick-derived isolates was 2.06°% (Yadav et al., 2019).

A phylogenetic and spatial diffusion analysis conducted in 2025 identified two major evolutionary clusters: Cluster A, originating in Karnataka and associated with the early outbreaks (1957-1972) as well as post-2010 re-emergence in Karnataka, Kerala, and Goa; and Cluster B, which spread from Maharashtra in the late 1970s into Tamil Nadu, Karnataka, and Kerala. The findings indicate a slow mutation rate consistent with long-term maintenance in sylvatic reservoirs rather than sustained human-to-human transmission. Spatial modelling estimated a median spread of approximately 59.7°km per year, with an evolutionary rate of ~10^{-4} substitutions per site per year (Paladan et al., 2025).

In addition, the main KFD vector, *Haemaphysalis spinigera*, exhibits genetic variability and population structuring. Differences among tick populations can affect their efficiency in acquiring and transmitting KFDV, thereby shaping local transmission dynamics. Such vector diversity indirectly influences the ecological context in which the virus evolves and may contribute to regional differences in viral maintenance and spread (Yadav et al., 2019).

6. Implications of KFDV genetic diversity for infections in humans

Current evidence indicates that the existing genetic diversity of KFDV has not substantially changed the core clinical presentation of the disease. Most viral isolates continue to produce a similar spectrum of symptoms in humans, characterised by fever, myalgia, and, in some cases, haemorrhagic manifestations.

Nevertheless, certain amino acid substitutions, particularly in Envelope-associated proteins, may influence viral replication, transmission efficiency, or host immune interactions. Genetic analysis of samples collected between 2018 and 2020 identified 28 non-synonymous (amino acid-altering) mutations, many located in the viral Envelope protein, a key determinant of host immune recognition and an important vaccine target (Sharma et al., 2025).

Specific molecular markers have been associated with potential pathogenicity. For example, positive selection has been observed at position 123 (A/T) in the E-protein, situated near the dimer interface essential for membrane fusion and viral infectivity (Yadav et al., 2019). Additionally, the presence of alanine at position 76 (in place of threonine) may destabilise the E-protein and increase the likelihood of unintended fusion events, which have been linked to haemorrhagic manifestations (Shah et al., 2019).

Despite these mutations, structural and antigenic analyses show no major alterations in the E-protein configuration in recent outbreaks. Consequently, no direct relationship has been established between these genetic variations and the detection of cases in newly affected geographical areas. This suggests that the virus may have been circulating earlier in several regions and that recent detection rather reflects improved surveillance, diagnostics, and awareness than true recent emergence. However, to clearly establish if there is a connection between genetic diversity and clinical symptoms more detailed studies would be required.

7. Knowledge gaps and future research priorities in understanding the genetic diversity and transmission dynamics of KFDV

Despite significant advances in sequencing and molecular epidemiology, important knowledge gaps remain in understanding the genetic diversity and its clinical implications as well as of KFDV. While this literature review consolidates current scientific knowledge; strategic investments in further studies would strengthen India's capacity to predict, detect, and respond to KFD outbreaks.

Based on the currently available data, the following challenges have been identified:

Limited longitudinal genomic data

Existing genomic information on KFDV remains sparse and geographically uneven. Most available sequences originate from a limited number of outbreak locations, predominantly in Karnataka, and consist of a combination of whole genome sequences and partial gene sequences, particularly of the E gene. This limits comprehensive understanding of the evolutionary dynamics and long-term circulation patterns of the virus across the Western Ghats region.

Incomplete understanding of phylogeography

While regional clustering of strains has been observed, the exact pathways of virus movement across forested landscapes remain unclear. To establish this, integrated studies combining viral genomics with wildlife ecology, livestock movement, human migration, land-use change and tick population genetics are required to map transmission corridors across the Western Ghats.

Role of wildlife hosts in viral maintenance

The contribution of different hosts to viral persistence and dissemination is still poorly defined. Linking host movement patterns with viral genetic variation could clarify how sylvatic cycles shape localized evolution.

Vector-virus interactions and tick genetics

Genetic variation within tick populations may influence viral acquisition, maintenance, and transmission efficiency, yet this interface remains scientifically underexplored. Comparative genomics of tick populations across endemic zones could explain micro-regional differences in outbreak dynamics.

Correlation between viral genetics and clinical symptoms

Although KFDV remains genetically conserved, subtle mutations, especially in E and NS proteins, may influence pathogenicity, immune response, or vaccine effectiveness. Robust clinical-genomic correlation studies are lacking and are essential to determine whether genetic variation affects disease severity or haemorrhagic manifestations.

Need for integrated One Health genomic surveillance

Current surveillance often operates in sectoral silos (human health, animal health, environment/ forest). A harmonized One Health sequencing framework, linking human cases, animal mortality, and tick surveillance, would enable real-time tracking of viral evolution and spread.

In conclusion, future research should move beyond isolated genetic studies towards integrated, landscape-level investigations that combine genomics, ecology, epidemiology, and clinical science to better predict and mitigate KFD emergence and transmission risks.

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