Tracing Zoonotic Bridges: A Phylogeographic Analysis of Nipah Virus (NiV) Outbreaks in India

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Abstract
Inthis study, 26 Nipah virus (NiV) genome isolates from various geographical pockets in India were employed to decipher the transmission and evolutionary mechanisms of NiV in the host and reservoir. A total of 24 unique haplotypes were identified in the course of this study, confirming the high genetic diversity of the viral genome. This study also demonstrates that infected human hosts and fruit bats (Pteropus medius) share overlapping virus haplotypes, which points to the virus's tendency to jump from bats to humans independently, rather than being continuously transmitted from an infected person to another in India. In geography, Kerala emerges as the focal reservoir of the virus that undergoes rapid evolution to give rise to different unique haplotypes. However, this study also reveals a surprising genetic similarity between Uttar Pradesh, Bihar, West Bengal, and Kerala, suggesting that the virus has had its share of long-range dissemination in the past, most likely through migratory fruit bats. This study also detects numerous gaps in the evolutionary network, implying that there are still many uncharacterized virus variants in the wild host reservoir. The findings of this study conclude that there is an urgent need for a genetic monitoring pipeline that tracks down the evolutionary and dissemination patterns of the virus at the human–wildlife interface before the next outbreak occurs.
Keywords: *Nipah virus; Median-joining network; Zoonotic spillover; Pteropus medius*

Introduction

Nipah virus (NiV) is one of the most notorious viruses that transmits from animals to humans. NiV is the member of family Paramyxoviridae that belongs to the genus Henipavirus responsible for significant considerable health issues in the population of South and Southeast Asia. For the first time, this virus was documented in 1998-1999 during the outbreak of Malaysia and Singapore. The NiV causes severeencephalitis and respiratory issues in domestic pigs, causing death of 100 human and also responsible for eradication of millions of pigs (Chua et al., 2000). In Malaysia, thepigs serve as a primary intermediate transmission vector (Paton et al., 1999). While, during the outbreaks in South Asia, NiV can transmit directly from wild animals to humans without intermediate host (Hsu et al., 2004). Several previously published studies confirmed that the fruit bat *Pteropus mediu*sis the natural host of NiV which is commonly,found throughout India (Sudeep et al., 2021). The South Asian lineage of NiV causes severe health issues like acute respiratory distress syndrome (ARDS) and intense encephalitis, with mortality rates ranging from 70%-100% (Mahalingam et al., 2012).

Throughout India, the NiV outbreaks show irregular patterns with long intervals. From India, the virus was documented from Siliguri, West Bengal, in 2001, where it spread rapidly through hospital facilities (Chadha et al., 2006), with persistent activities in the region like Nadia, West Bengal in 2007(Bandyopadhyay et al., 2010). For the long time, the researchers thought that the NiVmay restricted to northern and eastern regionsonly but themajor outbreak also documented from Kozhikode, Kerala in 2018, considered as the first record of NiV in south India (Arunkumar et al., 2019).After this instance, repeated cases from Kerala were recorded with geographically restricted outbreaks and autochthonoustransmission events from fruit bat populations across Western Ghats(Yadav et al., 2018). The cross-species transmission occurred through consumption of unprocessed date palm sap contaminated with saliva, urine, or faecalmaterial from infected Pteropus bats (Luby et al., 2009). Another reason for infection is handling fruits partially consumed by bats or visiting bat roosting sites (Plowright et al., 2015). Despite this, continuous human-to-human transmission was the major reason of outbreak

progression. The secondary infections arise due to respiratory droplet and other body secretions (Gurley et al., 2007). At the molecular level, viral envelope proteins mediate transmission of virus across different hosts by binding this conserved host cell-surface receptors like ephrin-B2 and ephrin-B3(Pernet et al., 2014). It is the most challenging task to explain the molecular variation and geographic distribution of these variants throughout India. Conventional phylogenetic trees cannot alwaysdemonstratecomplex evolutionary processes, including mutations in spatially isolated populations or undetected mutations in wild reservoirs (Haritsha et al., 2022). Hence, to overcome these constraints, median-joining network is the method of choice, provides valuable insight in depicting complex evolutionary steps, resolving convergent mutations, and monitoring transmission across different hosts and geographic areas (Kumar et al., 2019). Using median-joining approach, geographically restricted outbreaks can be directly attributed to specific zoonotic spillover incidents, facilitating comprehensive genomic surveillance across India.

Materials and Methods

A total of 26 Indian Nipah virus (NiV) genomes from the NCBI Virus database were compiled. Only sequences with a minimum length of 18,000 base pairs were included to ensure data quality.The dataset was aligned using the MUSCLE alignment tool (Edgar, 2004). DnaSP V6 was used to identify and analyse nucleotide variations, which revealed 24 unique haplotypes.Finally, NETWORK10was used to construct a median-joining network(Bandelt et al., 1999).

Results and Discussion

The networks constructed in this study characterize the geographic expansion, molecular epidemiology, and evolutionary trajectories of NiV within India. Haplotype analysis revealed 24 distinct haplotypes among the 26 NiV reflecting significant genetic diversity in NiV isolates sampled and sequenced from India.Current phylogenetic network suggests that the virus undergoes rapid, region-specific evolution driven by spontaneous mutations and the stringent selective pressures (Kumar et al., 2019). localisedsubsequent emergence is generally responsible for novel spillover events from wildlife agentsrather than continuous human-to-human transmission pathways (Clayton, 2017). The network analysis also reveals the formation of location specific cluster formed by Haplotype 22 (H_22) in Kerala, containing three human-linked haplotypes. This cluster characterises localised outbreak which further intensified through rapid human-to-human transmission or shared exposure to a contaminated source, analogous to situations documented in Siliguri and Kerala, where direct contact accelerates cases of virus (Arunkumar et al., 2019; Chadha et al., 2006). The genetic information also displayed a sequential pattern of mutations, progressing directly from H_22 to H_23, and subsequently branching to H_20 and H_21. This evolutionary pathway indicates ongoing viral replication accompanied by cumulative mutational changes transmitted through consecutive human carriers (Gurley et al., 2007).

This network analysis also indicates that Nipah virus cases in India stem from multiple independent zoonotic spillover incidents from Pteropus medius fruit bats, rather than originating from a continuous human transmission event (Kumar et al., 2019; Yadav et al., 2018). This zoonotic spillover is driven by the highly conserved composition of host cell receptors ephrin-B2 and ephrin-B3. The structural homology within these receptors lowers the structural barriers for cross-species transmission, hence restricting the virus's capacity to establish sustained transmission between different mammalian hosts (Pernet et al., 2014). Geographically, despite Kerala acting as a distinct, localized epicentre of the outbreak (Yadav et al., 2018), the network analysis displays a close association of haplotypes originating from Uttar Pradesh, Bihar, West Bengal, and Kerala (Plowright et al., 2015). The genetic links between southern and northern/eastern isolates confirms significant dispersalof virus between these regions, supported by the migratory patterns of fruit bats across the Indian subcontinent (Plowright et al., 2015).

Figure 1. Phylogeographic median-joining network of 24 unique Nipah virus haplotypes in India. Panel (a) displays host-specific distribution, while panel (b) shows state-wise geographic distribution.

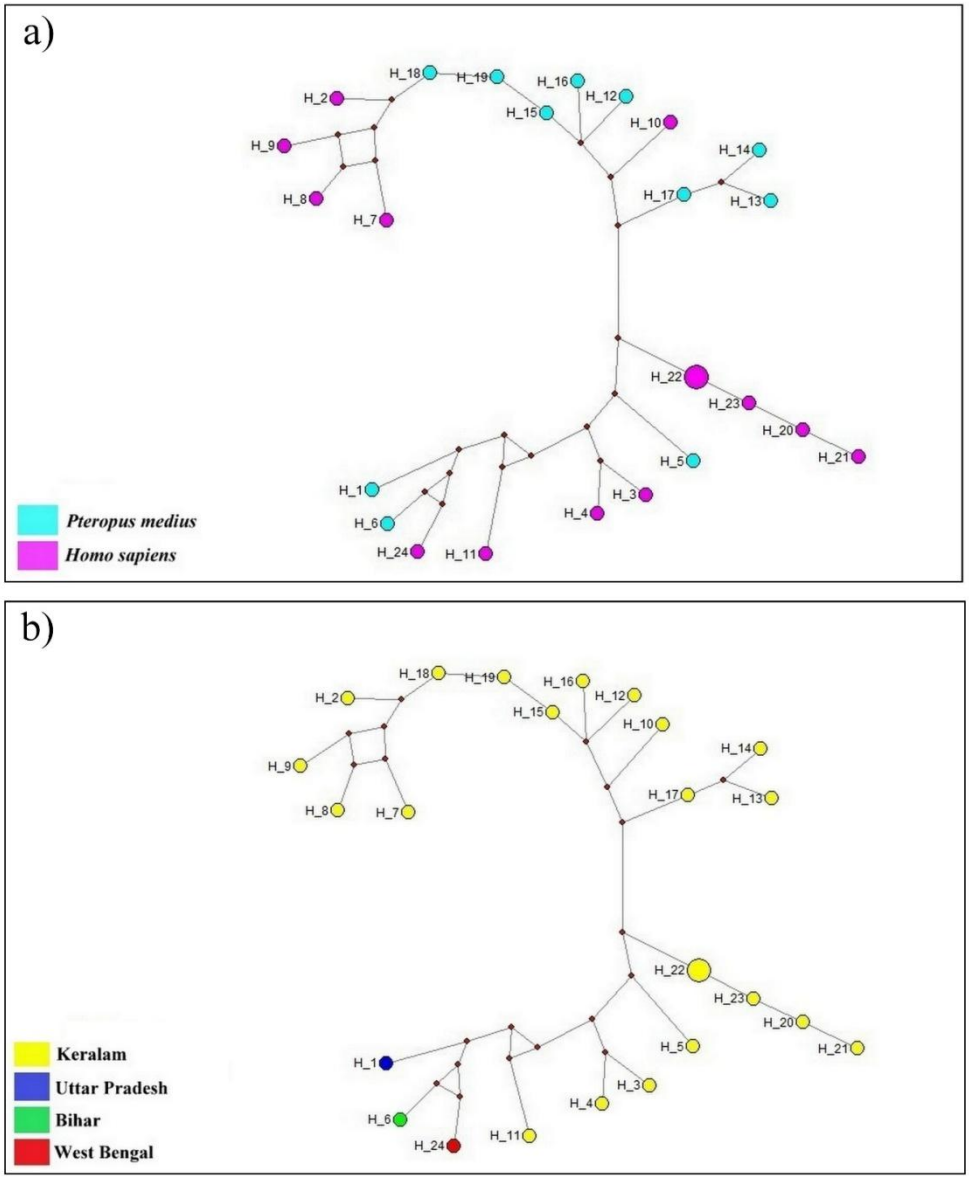


Table 1. Metadata and Repository Characteristics of the 26 Indian Nipah Virus (NiV) Genomic Sequences.

Sr. No.	Accession Numbers	Organism Name	Length in Base Pairs	Geographic Location
1	PX311054	*Henipavirus nipahense*	18193	Uttar Pradesh
2	PX242234	*Henipavirus nipahense*	18089	Keralam
3	PX242235	*Henipavirus nipahense*	18192	Keralam
4	PV640869	*Henipavirus nipahense*	18168	Keralam
5	PP554503	*Henipavirus nipahense*	18181	Keralam
6	PP554504	*Henipavirus nipahense*	18164	Bihar
7	OR820506	*Henipavirus nipahense*	18221	Keralam
8	OR820507	*Henipavirus nipahense*	18150	Keralam
9	OR820508	*Henipavirus nipahense*	18190	Keralam
10	OR130496	*Henipavirus nipahense*	18124	Keralam
11	OM135495	*Henipavirus nipahense*	18005	Keralam

12	MN549402	*Henipavirus nipahense*	18132	Keralam
13	MN549403	*Henipavirus nipahense*	18131	Keralam
14	MN549404	*Henipavirus nipahense*	18130	Keralam
15	MN549405	*Henipavirus nipahense*	18125	Keralam
16	MN549406	*Henipavirus nipahense*	18132	Keralam
17	MN549408	*Henipavirus nipahense*	18131	Keralam
18	MN549409	*Henipavirus nipahense*	18172	Keralam
19	MN549411	*Henipavirus nipahense*	18124	Keralam
20	MK336155	*Henipavirus nipahense*	18211	Keralam
21	MK336156	*Henipavirus nipahense*	18233	Keralam
22	MH523640	*Henipavirus nipahense*	18132	Keralam
23	MH523641	*Henipavirus nipahense*	18027	Keralam
24	MH523642	*Henipavirus nipahense*	18242	Keralam
25	MH396625	*Henipavirus nipahense*	18210	Keralam
26	FJ513078	*Henipavirus nipahense*	18252	West Bengal

Conclusion

Nipah virus cases in India originate from periodic cross-species transmission events between bat reservoirs and human populations, rather than persistent human-to-human transmission chains (Kumar et al., 2019; Yadav et al., 2018). Kerala functions as the primary hub for regional viral establishment and amplification, yet the virus's distribution across numerous states reveals a complex, interconnected epidemiological network encompassing northern, eastern, and southern territories with implications for the entire nation (Plowright et al., 2015). This context demands swift establishment of a holistic One Health surveillance infrastructure designed to monitor viral dynamics and evolution in both animal and human hosts across India (Bandyopadhyay et al., 2010).

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