*Neurology Asia 2009; 14 : 63 – 66*

# Nipah virus: Phylogeny and replication

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### *Abstract*

Phylogenetic analysis of Nipah virus isolates from Malaysia, Bangladesh and Cambodia suggested the presence of at least two different clusters of NiV strains. Based on the major glycoprotein (G) gene, the Nipah virus-Tambun isolate clustered with Nipah virus isolates from Cambodia and Bangladesh, whereas the remaining isolates from Malaysia clustered in a separate cluster. Sequence heterogeneity among the Nipah virus isolates from Malaysia was noted but the overall genomic sequence divergence value was small, suggesting a possible recent introduction of the virus. Nipah virus replicated well in porcine stable kidney cells and human lung fibroblast cells. Human monocytes, on the other hand were infected with Nipah virus but the cells did not support productive infection. Similarly, infection of human neuronal cells did not result in release of high infectious virus yield. The monocytes can serve to disseminate Nipah virus from site of infection including across the blood-brain barrier. And in the brain, Nipah virus is probably spread through cell-to-cell spread mechanism.

## INTRODUCTION

Nipah virus (NiV) is a zoonotic infectious virus responsible for an outbreak of severe and fatal encephalitis among pig farmers in Malaysia in 1998.[1] The outbreak was first reported in late September 1998 in Tambun (4° 37N, 101° 08E), near the town of Ipoh, Perak, Malaysia.[2] The disease spread southward within four to six months to several pig-farming communities around Seremban (2° 43N, 101° 57E), a town in Negeri Sembilan.[2,3] By April 1999, at least 85 human deaths have been reported in Seremban and 15 were recorded in Tambun.[4] The disease manifested in pigs as acute respiratory distress syndrome.[2,4,5] Infected humans, on the other hand, presented with fever, headache and drowsiness that could develop to fatal central nervous system infection in about 40% of the patients.[3,6] Humans contracted the infection most likely through contact with the excretions or secretions of NiV-infected pigs.[6]

Since the outbreak in Malaysia, NiV outbreaks have been reported in India[7,8] and Bangladesh.[9-17] To date, similar sporadic outbreaks have been reported almost annually in Bangladesh. The outbreaks, however, are not associated with infection of an intermediate host as only human showed evidence of NiV infection. Fruit bats or flying foxes, *Pteropus giganteus* are thought to be the source of NiV infection in Bangladesh.[15] Several studies conducted in Malaysia[18,19], Cambodia[20,21], Thailand[22,23], India[24], Madagascar[25] and West Africa[26] have shown evidences that bats are likely the natural reservoir for NiV. The bat population at several localities in these countries have been found to be seropositive for NiV, and NiV was successfully isolated from bats in Malaysia[19] and Cambodia[21] suggesting the strong likelihood that the initial outbreak of NiV in Malaysia also originated from infected bats.

## NIPAH VIRUS PHYLOGENY

The phylogenetics of NiV isolates from Malaysia[27-30], Bangladesh[31] and Cambodia[21] have been described. We aligned the open reading frames coding for the nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) and the polymerase protein (L) genes as described previously[30] for all NiV isolates available in the GenBank and examined the relationships of the viruses at the different genes. A very high degree of sequence similarity (>99%) between the NiV sequences of pigs, human and flying foxes from Malaysia were obtained. The phylograms constructed using N, P, M, F and L nucleotide sequences, showed a tight clustering of NiV isolates from Malaysia, while the NiV human isolate from Bangladesh and the flying foxes NiV from Cambodia diverged from this cluster (Figure 1A). At the G gene, however, slightly more heterogeneity was noted amongst the NiV isolates from Malaysia (Figure 1B). The pig isolate (NiV-Sungai Buloh), the human

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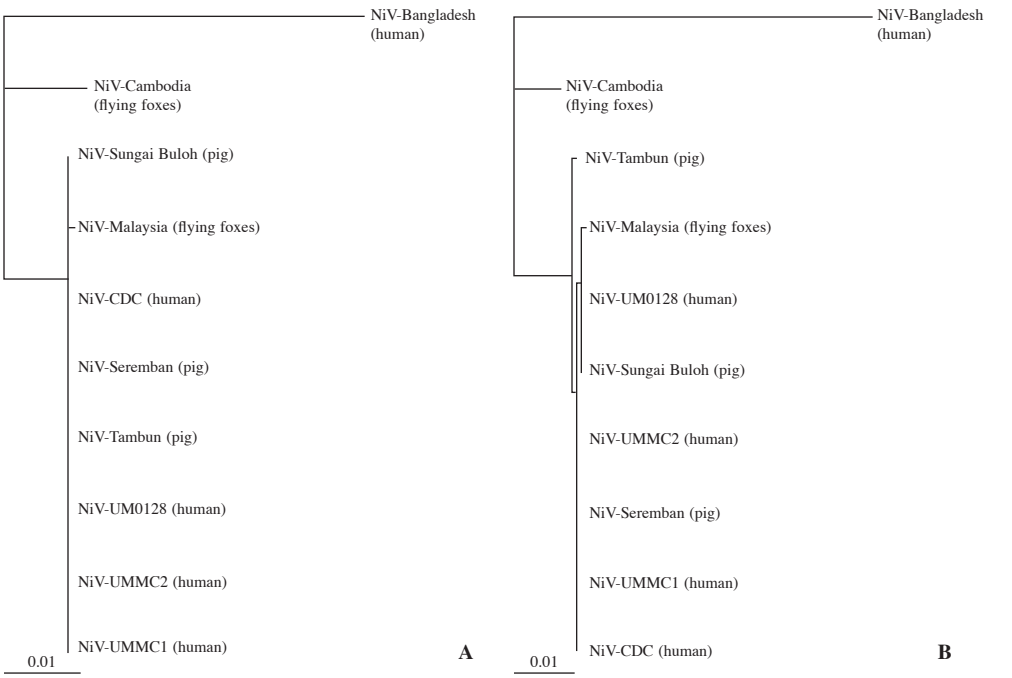


Figure 1. Phylogenetic relationship of the **A**) nucleoprotein (N); and **B**) glycoprotein (G) gene sequences of the Malaysian Nipah virus (NiV) isolates from pigs, human and flying foxes, the NiV human isolate from Bangladesh and the NiV isolate from flying fox in Cambodia. Abbreviations and accession numbers: NiV-Bangladesh, AY988601; NiV-Cambodia, AY858111; NiV-Tambun, AJ627196; NiV-Malaysia, AF376747; NiV-UM0128, AJ564623; NiV-Sungai Buloh, AJ564621; NiV-UMMC2, AY029768; NiV-Seremban, AJ564622; NiV-UMMC1, AY029767; and NiV-CDC, AF212302.

isolate (NiV-UM0128) and the NiV flying foxes isolate clustered closely, forming a cluster. The pig isolate, NiV-Seremban and the three other NiV human isolates, NiV-UMMC1, NiV-UMMC2 and NiV-CDC, formed the second cluster but the two clusters were not genetically distinct. The pig isolate, NiV-Tambun is the most diverged, forming a separate branch in a tight cluster of its own. The isolate shared higher G gene sequence similarity to NiV human isolate from Bangladesh and the flying foxes NiV from Cambodia than other NiV isolates from Malaysia. Subsequent analysis of the deduced amino acid sequences showed that three of the previously reported 11 amino acid changes in NiV-Tambun when compared to NiV-CDC are conserved changes in NiV human isolate from Bangladesh.[30] These conserved amino acid changes occur at position 147 of the M protein and at position 20 of the G protein, resulting in a residue change from serine to glycine and isoleucine to asparagine, respectively. A substitution of amino acid threonine for alanine at position 304 of the highly phosphorylated P protein may potentially reduce the phosphorylation site on the protein. These reaffirmed NiV-Tambun, a virus isolated from pig from the very first reported outbreak of NiV, as a different isolate from that which caused the most deaths in human during the Malaysia outbreak.[30] The higher sequence similarity of this virus to that reported from flying foxes in Cambodia and human cases from Bangladesh raised interesting questions about the potential origin of the index NiV outbreak and whether there were multiple, yet simultaneous NiV outbreaks in Malaysia in 1998 – 1999.[1-4] These findings suggest a potential of two main clusters of NiV strains, perhaps originating from two different populations of regionally circulating bats, with one in the Northern region of Peninsular Malaysia that include parts of the Indochina and the Indian subcontinent and the Southern region which include Indonesia and Papua New Guinea. The Northern region strains include NiV-Tambun and isolates from Bangladesh and Cambodia. Whereas, the remaining isolates from Malaysia formed the Southern region strains. Whether this is consistent with the *Pteropus* spp. flight paths requires further investigation.

In summary, the phylogenetic analysis of

the NiV isolates from Malaysia with the NiV isolates from Bangladesh and Cambodia further supports the possibility that the 1998 Malaysia NiV outbreak is unlikely to be due to a single transmission of NiV from bats to pigs. It also implies the possibility of other yet-to-be-isolated NiV strains that could exhibit more inter-strain sequence heterogeneity.

## NIPAH VIRUS REPLICATION

Nipah virus replication kinetics in the different host cells were examined to understand the pathobiology of NiV infection, and the possibility that the differences in the manifestations and severity of NiV infection in pigs and human were associated with the varying ability of the different cells to support NiV replication.[32,33] NiV-Sungai Buloh strain was used to infect four different cell types, one of pig (porcine stable kidney cells) and three of human origins (human neuronal cells, human lung fibroblasts cells and human monocytes). NiV replicated very well in the pig cells and human lung fibroblasts.[33] Infection of neuronal cells resulted in high accumulation of intracellular virus RNA but with low release of extracellular virus. Infection of human monocytes resulted in only low level of infection. Efficient infection of the pig cells was not surprising as pigs were the amplifying host for NiV during the outbreak in Malaysia.[5] In human infections, NiV is acquired mainly through aerosol or direct contact with infectious body fluid and secretions of infected pigs.[6] It is possible that the lungs are the initial target organ, from which the virus then spread to other organs. Its efficient replication in human lung fibroblast cells[33] suggest that NiV could replicate almost anywhere at the site of entry and from there on, infect other surrounding cells either through release of infectious virus particles or through the cell-to-cell spread mechanism.[34,35] Transmigration of infected monocytes across the blood-brain barrier could also be another route for NiV spread to the brain.[33] This is possible as NiV-infected monocytes were not rapidly destroyed by the infection enabling the infected monocytes to travel to other parts of the body including the brain to cause infection of the neurons. The neuronal cells, however, had the lowest ability to support productive NiV infection amongst the cells examined, suggesting that NiV do replicate in the neuronal cells but it is much less efficient and there is no immediate release of virus particles. A cell-to-cell spread mechanism is most likely for infection of the neurons and this could have contributed to the foci-like lesions consisting of giant syncytial cells seen in some of the infected brain tissues.[36] The possibility of a similar slow cell-to-cell spread mechanism involving the role of defective NiV in the relapse cases of NiV infection needs to be investigated.

In summary, NiV is a new zoonotic virus known to infect human. The virus could have been carried by bats and not caused infection in human until it accidentally infected an efficient amplifying host - pigs, from which human contracted the infection. The different bat population perhaps harbours many more NiV strains and as long as there is a chance for pigs to be infected with NiV, there is always a possibility that NiV outbreak could recur in human. There have been cases of NiV infection in human following exposure to bats-contaminated date palm juice[15] but in the absence of an amplifying host, the cases remained limited to those who directly consumed or are involved in processing of the tainted saps. This however, does not rule out the possibility that there are NiV strains that could be highly transmissible from human-to-human. Hence, continuing NiV surveillance is necessary and research in NiV that could lead to the development of vaccines needs to be given better emphasis.

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